A NEUROPHARMACOLOGICAL STUDY OF SOME ASPECTS OF CAROTID BODY CHEMORECEPTOR ACTIVITY IN THE CAT

by

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CONTENTS

DECLARATION

Statements in terms of Ph.D. regulations 2.4.15 of the University of Edinburgh.

1. I hereby state that this thesis has been composed by me and is based on work carried out by myself, either alone or in collaboration with my supervisor, Dr. D.S. McQueen. I contributed substantially to the experimental design, surgical procedures, calculations, analysis and subsequent interpretation of results.

2. Estimations of PO_2, PCO_2 and pH in samples of arterial blood were performed by Mrs. S. Bond. The computer programmes used on the PET 32K microcomputer for data analysis were devised by Dr. H.M. Brash (Department of Medicine, University of Edinburgh) and Mrs. Bond.

J.A. Ribeiro

Statement in terms of Ph.D. regulation 2.4.11 of the University of Edinburgh.

The results of some experiments reported in this thesis have been published (see Appendix I) or are awaiting publication, and are related to:

Section III:

1. McQUEEN, D.S. & RIBEIRO, J.A. (1981). Excitatory action of adenosine on cat carotid chemoreceptors. *J. Physiol.*, 315, 38-39P.

2. McQUEEN, D.S. & RIBEIRO, J.A. (1981). Effect of adenosine on carotid chemoreceptor activity in the cat. *Br. J. Pharmac.*, 74, 129-136.

3. RIBEIRO, J.A. & McQUEEN, D.S. (1982). On the neuromuscular depression and carotid chemoreceptor activation caused by adenosine. In: *Physiology and Pharmacology of Adenosine Derivatives*, ed. Daly, J.W., Phillis, J. and Kuroda, Y. (in press). New York: Raven Press.

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1. McQUEEN, D.S. & RIBEIRO, J.A. (1980). Inhibitory actions of methionine-enkephalin and morphine on the cat carotid chemoreceptors. *Br. J. Pharmac.*, 71, 297-305.

2. McQUEEN, D.S. & RIBEIRO, J.A. (1981). Comparison of the depressant effects of leucine- and methionine-enkephalin on spontaneous chemoreceptor activity in cats. *Br. J. Pharmac.*, 72, 544-545P.

3. McQUEEN, D.S. & RIBEIRO, J.A. (1981). Effects of β-endorphin, vasoactive intestinal polypeptide and cholecystokinin octapeptide on cat carotid chemoreceptor activity. *Q. Jl exp. Physiol.*, 66, 273-284.

Section V:

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ABSTRACT

Afferent chemoreceptor activity was recorded from the peripheral cut end of the carotid sinus nerve in pentobarbitone anaesthetized cats. The effects of purines, peptides and ouabain on chemosensory activity were studied.

Purines. It was found that intracarotid injections of adenosine; AMP; ADP; ATP; CoA; the adenosine analogues: N^6-methyladenosine, 2'-chloroadenosine, 3'-deoxyadenosine but not 2'-deoxyadenosine; cyclic AMP; dibutyryl cyclic AMP increased spontaneous chemoreceptor discharge. The ATP analogues, α-β-methylene ATP decreased spontaneous chemoreceptor discharge, whereas the β-γ-methylene ATP caused a slight increase in discharge. Adenine and the purine nucleosides inosine and guanosine had little or no effect on the discharge. The pyrimidine nucleosides cytidine and uridine were also studied and had little or no effect on spontaneous chemoreceptor discharge. Intracarotid injection of theophylline transiently depressed spontaneous chemosensory activity and potentiated the action of adenosine. Intracarotid injection of dipyridamole increased spontaneous chemoreceptor discharge and the chemoexcitation evoked by low doses of adenosine and ATP was potentiated whereas that caused by high doses was inhibited and associated with a decrease in arterial blood pressure. Dipyridamole administered intravenously increased the chemoexcitatory actions of both low and high doses of adenosine. Responses evoked by sodium cyanide were slightly and variably modified during an adenosine infusion and those evoked by acetylcholine and dopamine were increased. It is concluded that adenosine increases chemoreceptor activity by acting on an extracellularly located receptor, which is theophylline-insensitive and probably of the R-site type.

Peptides. The opioid peptides, methionine-enkephalin, leucine-enkephalin and β-endorphin inhibited spontaneous chemoreceptor discharge. β-endorphin was a less potent inhibitor and the inhibition it evoked was very similar to that of morphine. Chemoinhibition induced by β-endorphin was greatly reduced by naloxone; the inhibition associated with the enkephalins was also decreased, although not so markedly. Vasoactive intestinal polypeptide in low doses decreased spontaneous chemoreceptor activity whereas higher doses of the same substance increased chemoreceptor activity as did cholecystokinin octapeptide. Substance P was unable to overcome the chemoinhibitory effect of methionine-enkephalin. It is concluded that peptides such as methionine-enkephalin, leucine-enkephalin and vasoactive intestinal polypeptide, known to be present in the carotid body as well as others present in the brain, such as β-endorphin and cholecystokinin octapeptide, influence carotid body chemoreceptor activity. The opioid peptides act via naloxone-sensitive receptors.

Ouabain. Ouabain increased spontaneous chemoreceptor activity. During infusions the excitation was followed by a decline in discharge to frequencies near the control level. During the excitation the stimulatory action of sodium cyanide, carbon dioxide-equilibrated Locke solution and acetylcholine were potentiated, as was the chemoinhibition induced by dopamine. During the post-excitatory phase the responses evoked by these substances were reduced or abolished. It appears that ouabain has two distinct actions on the carotid body chemosensory activity, a 'sensitizing' followed by a 'desensitizing' phase.

The possibility that the various substances investigated act via a common mechanism (e.g. adenylate cyclase-cyclic AMP system) is discussed.

LIST OF ABBREVIATIONS

The abbreviations listed below are used at various points in the text but not in headings. Some of the abbreviations are introduced in the text and are listed here for convenience of the reader.

ACh	acetylcholine
ADP	adenosine 5'-diphosphate
AMP	adenosine 5'-monophosphate
ATP	adenosine 5'-triphosphate
ATPase	adenosine triphosphatase
B.P.	arterial blood pressure
cyclic AMP or cAMP	adenosine 3', 5'-monophosphate
cyclic GMP	guanosine 3', 5'-monophosphate
Db-cyclic AMP	dibutyryl cyclic AMP
DMPP	dimethyl-4-phenylpiperazium
g	gram
5-HT	5-hydroxytryptamine
i.c.	intracarotid
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
kg	kilogram
leu-ENK	leucine-enkephalin
m	metre
met-ENK	methionine-enkephalin
mg	milligram
min	minute
ml	millilitre
mm	millimetre
mmHg	millimetres of mercury
ms	millisecond
µg	microgram
µm	micrometre
NA	noradrenaline
NaCN	sodium cyanide

ng	nanogram
nm	nanometre
nmol	nanomole
pmol	picomole
s	second
s.e. mean	standard error of mean
SIF	small intensively fluorescent
SP	substance P
Torr	torr
VIP	vasoactive intestinal polypeptide

SECTION I

General Introduction

This thesis explores the actions of purines, neuropeptides and ouabain on the cat carotid body chemoreceptors using electrophysiological techniques to record chemoreceptor action potentials from the carotid sinus nerve in order to provide a measure of chemoreceptor activity.

The carotid body is a sensory organ (De Castro, 1928, 1940) and its chemoreceptor function definitively proven (Heymans, Bouckaert and Dautrebande, 1930; Schmidt, 1932). One of the properties of its chemoreceptors is the capacity to generate afferent impulses at a frequency dependent on the PO_2, PCO_2 and the pH of the blood perfusing it, and so to initiate the necessary cardiovascular and respiratory reflexes to correct any changes observed in those factors. For example, stimulation of the carotid body chemoreceptors causes (1) increase in the tidal volume, frequency and minute volume of breathing (Heymans, Bouckaert and Dautrebande, 1930; Heymans and Bouckaert, 1933); (2) systemic vasoconstriction (Daly and Ungar, 1966) and bradycardia (Daly and Scott, 1958); (3) increase in bronchiolar tone (Daly and Schweitzer, 1951); (4) increase in the pulmonary vascular resistance (Daly and Daly, 1959); (5) increase in secretion of the adrenal medulla and cortex (Anichkov, Malyghina, Poskalenko and Ryzhenkov, 1960); (6) increase in activity of the motor cortex, leading to convulsions (Schmidt and Comroe, 1940).

Chemoreceptors are activated by a fall in PaO_2 or pH and by an increase in $PaCO_2$, with the main natural stimulus being hypoxia: the lower the local tissue PaO_2 at the receptor, the greater the chemoreceptor stimulation (Biscoe, Purves and Sampson, 1970).

In the usual laboratory animals (e.g. dogs, cats, rabbits, rats) the carotid body is a small structure located bilaterally on the root of

the occipital artery or on the common trunk of the occipital and ascending pharyngeal arteries from which receives the blood supply (see Adams, 1958) (see also Figure 2.1).

1.1 Historical

The progress in the knowledge of the carotid body has been associated with the vicissitudes of the progress of biological sciences.

A comprehensive history of the carotid body told through the eyes of a morphologist was written by Adams (1958). Interesting controversies can be detected since the discovery of the carotid body in 1742 by Berckelmann (cit. by Adams, 1958) who was pupil of Haller. For an account on the history of the discovery of the carotid body see also Pick (1959).

In the early literature, carotid body nomenclature was related to the interpretation of the putative function of the organ. For example, ganglion minuten (Taube, 1743 cit. by Pick, 1959), ganglion exigum (Haller, 1762 cit. by Adams, 1958 and Pick, 1959), ganglion parvum (Neubauer, 1772 cit. by Adams, 1958); ganglion intercaroticum (Andersh, 1797; Mayer, 1833; Valentin, 1833 all these authors cit. by Adams, 1958 and Pick, 1959) were related to the interpretation that the carotid body was a sympathetic-like ganglion. In 1862 Luschka (cit. by Adams, 1958 and Pick, 1959) considered the carotid body as a "nerve-gland" like the adrenal gland, the anterior pituitary or the coccygeal gland and named it glandula carotica. Three years later, Arnold (1865) stressed its vascular richess and suggested the name glomeruli arteriosi intercarotici. In 1892, Stilling (cit. by Adams, 1958) proposes a reconciliation between the "gland" school and the "vascular" school and suggested the name paraganglion. Paraganglion intercaroticum was the name adopted by Kohn (1900), Vincent (1922) who

considered it as a gland of internal secretion, and De Castro (1926). Another term was suggested still later by Celestino da Costa (1939, 1940, 1954) that of metaneurogonia on the grounds that the carotid body is composed of "elements neuraux ... qui s'y differencient assez peu eu régle" and that some of the cells may have similarities with Schwann cells.

Basically, the controversy was between the carotid body being a sympathetic-like ganglion as its neighbours, the superior cervical ganglion or the nodose ganglion or alternatively, a kind of endocrine gland similar to the adrenal medulla. In the polemics the embryological nature of the carotid body, ectodermic or mesodermic (see e.g. Adams, 1958; Kondo, 1975) was frequently invoked either to support the "ganglion" or the "gland" schools or whenever attempts were made to reach a compromise between both schools. The basis of the compromise was that the carotid body shares properties of both neural ganglia and the adrenal medulla. Recently the same notion has been revived in the Amine Precursor Uptake and Decarboxylation (APUD) (Pearse, 1969) and the paraneurone (Fujita, 1977, 1980) concepts. The carotid body type I cells can be classified as being members of both groups. Both theories seek to break down the barriers that exist between nerve and gland cells which as the poetic expression of Fujita and Kobayashi (1979) says, "neurones and paraneurones are continuous like the colours of the rainbow". The central dogma of the paraneurone concept, as Kobayashi and Fujita (1981) pointed out recently, is the 'continuity' of neurones and endocrine/sensory cells. These cells are considered as forming a continuous spectrum with regard to structure (neurosecretion-like and/or synapse-like granules), products (peptides, amines and adenine nucleotide secretion common to or closely related to neurohormones

and neurosecretions) and function (recepto-secretory). Carotid body type I cells are of the 'closed' type because they are closed in the internal environment and receive their main stimulation from the blood stream.

The APUD concept was advanced by Pearse (1969) to draw together under the same umbrella endocrine and nerve cells. According to a recent account (Pearse, 1978), the APUD concept is now expressed in the following terms: "the cells of the APUD series, producing peptides active as hormones or as neurotransmitters, are all derived from neuroendocrine-programmed cells originating from the ectoblast. They constitute a third (endocrine or neuroendocrine) division of the nervous system whose cells act as third-line effectors to support, modulate or amplify the actions of neurones in the somatic and autonomic divisions, and possibly as tropins to both neuronal and non-neuronal cells". The author divided the functions of the APUD cells into six categories according to the site where they secrete (1) neurocrine (into neurones), (2) neuroendocrine (via axons), (3) endocrine (into the bloodstream), (4) paracrine (into intercellular space), (5) epicrine (into somatic cells), (6) exocrine (to the externum). The SIF and carotid body type I cells are neurocrine.

1.2 Structure and function

Much of what is known about the morphology of the carotid body stems from discoveries by De Castro (1926, 1928) who established that the main innervation of the carotid body has its origin from the glosso-pharyngeal nerve and not from the sympathetic. De Castro (1928) was the first to demonstrate the sensory innervation and the sensory function, but it was Heymans and colleagues (see Heymans and Neil,

1958 and references therein) who extensively studied the reflexes arising from the carotid bifurcation and identified the chemoreceptor reflexes associated with the carotid body.

It is generally accepted that in all mammals studied to date the parenchyma of the carotid body includes a glomus complex containing type I and type II cells, nerve endings terminating on glomus cells and blood vessels (see Figure 1.1). Connective tissue exists between and around the glomus complexes. Myelinated (A) and unmyelinated (C) fibres from the carotid sinus nerve are also present. Myelinated fibres (dimaters of 1-10 μm, conduct at 4-53 m/s) represent about two-thirds of the total number of chemoreceptor fibres, the other third being unmyelinated (0.1 - 1.3 μm diameter, 0.5 - 2.0 m/s) (Fidone and Sato, 1969; see also Eyzaguirre and Fidone, 1980). A single sensory fibre usually branches extensively to innervate between 10 and 20 type I cells, and according to Eyzaguirre and Gallego (1975) and Kondo (1976) this may constitute the sensory unit. The existence of myelinated and non-myelinated fibres may be related to conduction of different information (see Biscoe, 1971). For a review of recent controversies on the carotid body morphology including its inherent semantic problems see Biscoe (1971).

Assigning function to morphology, one can say in a rather condensed way:

1.2.1 Type I cells

The type I cells seem to operate as chemoreceptor cells. By this interpretation carotid body chemoreceptors have traditionally been considered to be composite or secondary receptors, i.e. receptors where a non-neural element is the primary target or receptor of the stimulus. This implies that a sensory or afferent synapse should operate

De Castro (1951)

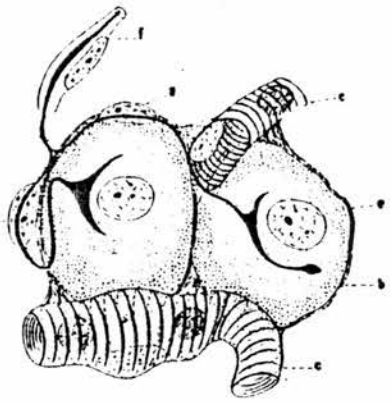
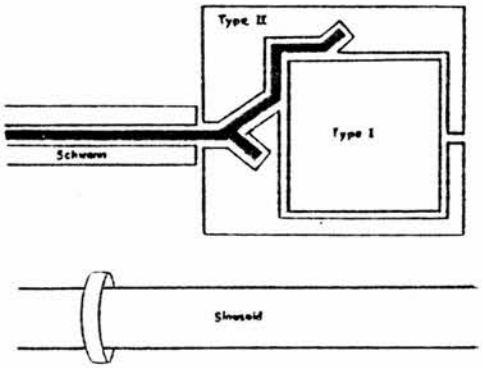


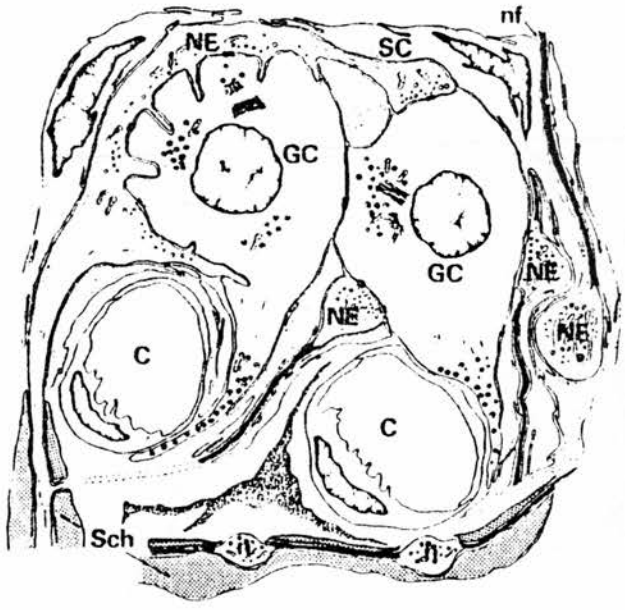
Diagram showing the structure of the synapse in the chemoreceptors. The epithelioid cells, *e*, present a considerable surface area in close relationship with the blood vessels (*c*). *f* = sensory nerve fibre which possesses a myelin sheath; *a* Schwann cell which encloses the unmyelinated nerve fibres which form the "menisques terminaux"; *b* = connective tissue network.

Torrance (1968)



Grossly oversimplified diagram of cells of chemoreceptor tissue. A Type I cell is surrounded by a Type II cell and its processes. A nerve fibre leaves a Schwann cell to be enveloped in a Type II cell. Its terminals may, or may not, come to lie up against the Type I cell. A sinusoid, with muscle around the vessel afferent to it, lies close to the Type I and II cells.

Nishi (1976)



Schematic representation of the chemoreceptor system of the carotid body in the ca
GC: glomus cell, presumably chemoreceptor cell. NE: afferent nerve ending nf: nerve fiber n: nerv
ending, presumably sympathetic in origin. C: capillary

FIGURE 1.1

Schematic representation of the main structural components of the carotid body according to De Castro (1951), Torrance (1968) and Nishi (1976). Note that in De Castro's representation based on light microscopy the nerve endings penetrate the glomus cells, and in Torrance's scheme that type II cell envelops the carotid sinus nerve axon as it leaves its sheath to its termination in contact with type I cell also surrounded by the type II cell. In the Nishi's scheme all structural components of the carotid body are contemplated: type I cells (GC), type II cells (SC), carotid sinus nerve endings (NE) and fibres (nf), capillaries (c), nerve endings presumably of sympathetic origin (n) and Schwann cells enveloping both carotid sinus nerve and presumably sympathetic fibres (Sch).

The type I cells contain catecholamines (dopamine, noradrenaline, adrenaline), 5-HT and perhaps ACh. They also contain neuropeptides (met-ENK, leu-ENK, SP and VIP) and ATP.

between the type I cell and the sensory nerve endings. The type I cells are ovoid cells about 10 μm diameter with large nuclei, well-developed mitochondria, endoplasmic reticulum, Golgi apparatus and numerous dense-core vesicles (30 - 120 nm) which are similar to but smaller than those present in the adrenal medulla (e.g. Lever, Lewis and Boyd, 1959; Hellstrom, 1975; Verna, 1979); they occur in groups of two or three surrounded by a type II cell (see Figure 1.1). Fluorescence microscopy, electron microscopy, immunohistochemistry and chemical analysis have revealed the presence of catecholamines (dopamine, noradrenaline, adrenaline) and 5-hydroxytryptamine (Chiocchio, Biscardi and Tramezzani, 1966; 1967), neuropeptides: methionine-enkephalin (met-ENK), leucine-enkephalin (leu-ENK), vaso-active intestinal polypeptide (VIP), substance P (SP) (Lundberg, Hökfelt, Fahrenkrug, Nilsson and Terenius, 1979; Cuello and McQueen, 1980; Wharton, Polak, Pearse, McGregor, Bryant, Bloom, Emson, Bisgard and Will, 1980; Fitzgerald, Raff, Garger, Fechter, Anand and Said, 1981) and ATP (Böck, 1980). Using a bioassay method, i.e. injecting carotid body extracts and measuring the arterial blood pressure in cats Eyzaguirre, Koyano and Taylor (1965) detected a high level of ACh-like material in the cat carotid body; this amount has been reduced to one-tenth of the bioassay measurement by using chromatography/mass fragmentometry (≃ 12 nmol/g tissue ~ 12 pmol/organ) (Fidone, Weintraub and Stavinoha, 1976). However, there is still no direct evidence that the ACh detected is stored in type I cells. In spite of the lack of unequivocal morphological evidence to support the existence of two or more varieties of type I cell (see Biscoe, 1971) the recent observations that type I cells can contain ATP and a number of neuro-peptides as well as catecholamines, 5-HT and possibly ACh and that all

these substances may be contained in vesicles make it plausible that different type I cells may exist with different combinations of those substances. The presence of some non-innervated type I cells (see Eyzaguirre and Fidone, 1980) may be related to a different function for these cells with respect to type I innervated cells.

1.2.2 Type II cells

The type II cells are supporting or sustentacular cells similar to the Schwann cells of the motoneurones or the glial cells. They support the type I cells and surround the carotid sinus nerve axon in its passage from its sheath to its termination on the surface of the type I cell (see Comroe, 1964) (see Figure 1.1). The type II cells contain a nucleus and the usual cytoplasmic inclusions (Golgi apparatus, endoplasmic reticulum, mitochondria and fibrils). It is possible that ACh is stored in these cells (Biscoe, 1971; Eyzaguirre and Fidone, 1980) as has been shown to be the case in the Schwann cells of the motor neurones of denervated preparations (Birks, Katz and Miledi, 1960; Miledi and Slater, 1968). Schwann end-plate potentials and Schwann miniature end-plate potentials follow Poisson statistics, i.e. random release characteristics (Bevan, Grampp and Miledi, 1976) are blocked by curare and are independent of Ca^{2+} (Dennis and Miledi, 1974).

1.2.3 Nerve endings

Carotid sinus nerve fibres terminate on glomus cells in a variety of nerve-ending formations, including calices, boutons, and a number of intermediate forms. Some of the nerve endings are relatively large (up to 15 μm diameter). A nerve filament may branch from one ending to innervate a type I cell, with a second ending terminating as a calyx, a bouton, or an intermediate form on another type I cell (De Castro,

1940; Nishi, 1976). The carotid sinus nerve endings can be divided into afferent (sensory) and efferent (motor). The zones of apposition between endings and cells are separated by a cleft of 20 - 30 nm and a number of a small (30 - 40 nm) clear-core vesicles are present in the nerve endings and sometimes in the glomus cells as well. Synaptic-like membrane densifications have been detected on one or both sides of the junction. According to the location of these electron-dense zones and accumulations of vesicles, the nerve endings have been classified as presynaptic, postsynaptic or reciprocally synaptic to the type I cells. This picture varies with different species (see Eyzaguirre and Fidone, 1980) and much may depend on the methods used for preparing the tissues. The presence of efferent synapses between pre- or post-ganglionic sympathetic fibres (from the superior cervical ganglion) and the glomus cells, has also been reported (e.g. Verna, 1971; McDonald and Mitchell, 1975).

Since chemosensory activity is depressed when the carotid sinus nerve is stimulated electrically it has been suggested that an efferent pathway exists in the nerve (see Biscoe and Sampson, 1968; Neil, and O'Regan, 1971). Sympathetic nerve endings and fibres (most of them unmyelinated - Eyzaguirre and Uchizono, 1961) from the superior cervical ganglion are also present in the carotid body and they appear to be involved in regulating blood flow. In the cat with the carotid body sympathetically denervated the blood flow is about 38 mm^3/min compared with its value (82 mm^3/min) before ganglionectomy (Daly, Lambertsen and Schweitzer, 1954). The existence and function of fibres coursing from the vagus nerve or from the nodose ganglion to the carotid body has yet to be established (see Biscoe, 1971).

1.2.4 Blood vessels

The arteries divide into fenestrated capillaries which are in close contact with type I cells, and supply the blood to be 'tasted' (see Torrance, 1968) by the chemoreceptors for its PaO_2, $PaCO_2$ and pH; the veins draining carotid body pass either directly into the jugular vein or into the transverse vein and then into the external or internal jugular veins (see Comroe, 1964). Blood flow through the carotid body is very high (about 2 l/100 g of carotid body tissue/min) (Daly, Lambertsen and Schweitzer, 1954), the total venous outflow from the carotid body arises from the tissue vascular bed plus that which is diverted through an arteriovenous shunt whenever this is present (De Castro, 1951; Seidl, 1975).

Despite type I cell being regarded as a chemoreceptor cell, existence at the nerve endings of a regenerative region which is sensitive to a number of stimuli, e.g. NaCN, ACh, has also been proposed (see Paintal, 1977). So the possibility of fine nerve endings being chemoreceptors as proposed by Biscoe (1971) partly on the basis of experimental evidence (the chemoreceptor afferent fibres have a discharge pattern which is compatible with a Poisson process) but mainly on theoretical grounds (fibre dimensions and geometry; K^+ depolarization calculated by the Nernst equation; ATP productions either taking place near the pumping (Na^+, K^+-ATPase) site or diffusing rapidly to that site; oxygen consumption and high energy phosphate bonds formation; localization of a low-affinity cytochrome as a source of energy bypassing ATP), has been sustained experimentally by others. For instance, neuromas of the sinus nerve (without glomus type I or II cells) respond to low PaO_2 and high $PaCO_2$ (Kienecher, Knoche and Bingmann, 1978; Tan, Pallot and Purves, 1981). A compromise may be

that both glomus cells and nerve endings function as chemoreceptors, their relative importance being dependent upon the type of stimulation. The information carried centripetally may also depend on the type of fibre (A or C) used.

1.3 Methods of studying the carotid body chemoreceptor pharmacology

Information about the effects of drugs on chemoreceptors can be obtained by studying the cardiovascular and respiratory reflexes originated in the carotid body or in a more direct way by recording bioelectrical activity from the carotid sinus nerve. The latter can be performed *in vivo* or *in vitro*.

1.3.1 Reflex studies

These include effects on respiratory and cardiovascular reflexes obtained by stimulating the chemoreceptors following administration of drugs in the whole animal.

The methods used in early studies provide only limited information about the action of drugs on carotid body chemoreceptors. For example, secondary actions (e.g. in blood gas tensions, lung inflation reflex, arterial blood pressure) which can modify the primary chemoreceptor reflex might mask the chemoreceptor action of the drug. Moreover, integration in the central nervous system makes it difficult to relate output (reflex change) to input (drug action on chemoreceptors). The main advantages of the method are: (1) it is easy to perform; (2) it provides some information concerning the actions of drugs and physiological stimuli on the whole intact receptor populations (see Heymans and Neil, 1958; Anichkov and Belen'Kii, 1963).

1.3.2 Electrophysiological studies

The use of electrophysiological techniques have as their main advantages: (1) the possibility of directly measuring the electrical activity of neurones; (2) they have a high temporal resolution which helps to relate cause and effect; (3) they can be used to provide quantitative data.

1.3.2.1 In vivo recordings:

The advantages of this technique are: (1) the cells are more or less close to their milieu interieur; (2) if anaesthesia and other experimental factors such as B.P., blood gases (PaO_2 and $PaCO_2$) and pH are controlled then the action of drugs may be studied in the carotid body under relatively physiological conditions. The most used technique consists in obtaining recordings from the carotid sinus nerve previously cut near the point where it joins the glossopharyngeal nerve. By this method it is possible to eliminate any efferent activity of the carotid sinus nerve (see e.g. Bogue and Stella, 1935; Heymans and Neil, 1958; McQueen, 1977). A refinement of this technique is to record from a single chemoreceptor unit since in this condition there is no doubt when a unit dies, as pointed out by Biscoe (1971). Another technique consists of obtaining recordings from the petrosal ganglion; this allows one to maintain carotid sinus nerve efferent influence to the carotid body (see Vidruk and Dempsey, 1980).

In both methods it is possible to eliminate the ganglioglomerular (sympathetic) activity. The limitations of these methods arise from the fact that it is not possible to know where within the receptor complex a drug is acting and the sample of fibres studied is limited. Vascular effects of drugs may complicate interpretation of results.

1.3.2.2 In vitro recordings:

These techniques include extracellular and intracellular recordings and offer the advantage of permitting the application of drugs in precisely known concentrations, and avoiding vascular complications. Other advantages are the exclusion of anaesthesia and the possibility of elucidating the ionic mechanisms involved in responses. The main disadvantages in relation to the carotid body are the absence of blood, the drugs take longer to act, and their effects last longer which contrasts with the dynamic characteristics for the carotid chemoreceptors *in vivo*.

- *Extracellular recordings:*

This technique hss been largely used by Eyzaguirre's group (see e.g. Eyzaguirre and Lewin, 1961; Eyzaguirre and Zapata, 1968a; Eyzaguirre and Nishi, 1974). The technique allows the recording of chemoreceptor discharge as *in vivo* or the recording of the mass receptor potential which is taken as a cumulative measure of the polarization of sensory nerve endings in the carotid body (see Eyzaguirre and Nishi, 1974).

- *Intracellular recordings:*

This technique improves the proximity to the receptor and creates the possibility of administering drugs into cells. However, the membrane potentials recorded (10 - 60 mV with a mean of 20 mV) are much lower than those reported for excitable tissues (Gallego and Eyzaguirre, 1978). The main disadvantage of intracellular recordings is that damage to cells is caused by the relatively gross microelectrodes commonly used. Sample bias arises because it is more likely that larger rather than smaller cells and nerve fibres will be impaled by the

microelectrodes (see Eyzaguirre and Fidone, 1980). Another difficulty arises from the fact that many type I cells are covered by processes of type II cells and so the information obtained may be a combination of effects involving the membranes of both cells.

1.3.3 Various methods

These include tissue culture technique which enables a correlation of microelectrophysiological and biochemical experiments on a relatively homogeneous population of cells (see e.g. Acker and Pietruschka, 1977). Other methods are related to bioassay, biochemistry, immuno-histochemistry, histofluorescence, electron microscopy, the use of gas-chromatography - mass-spectrometry and binding studies.

1.4 Pharmacology

Carotid body chemoreceptors can be stimulated by a large number of chemical substances - about 70 compounds were listed by Heymans and Neil (1958). This kind of 'omnireceptor' (Eyzaguirre and Fidone, 1978) can be stimulated by (1) chemicals known to modify utilization of oxygen in tissues by interfering with cytochrome oxidase. Sodium cyanide is the best example and the most frequently used; (2) chemicals known as nicotinic agonists. This group includes nicotine, ACh, DMPP; (3) miscellaneous substances including ATP, veratridine, papaverine, potassium ions (see Heymans, 1955; Comroe, 1964).

The role of substances present in the carotid body which are capable of modifying chemoreceptor activity when exogenously applied to the carotid body, such as ACh and catecholamines, has been investigated in recent years. Despite lack of definitive evidence that chemical transmission of sensory impulses occurs in the carotid body

(see reviews by Torrance, 1968; Bisooe, 1971; Eyzaguirre and Fidone, 1980) ACh and catecholamines (in particular dopamine) fulfill some of the requirements of putative carotid body transmitters. A brief review of some of the evidence follows.

1.4.1 Acetylcholine and catecholamines

- *Acetylcholine*

The discovery of ACh chemoreceptor stimulatory properties led to the idea that the substance might be an excitatory transmitter in the carotid body. This was first suggested by Schweitzer and Wright (1938) on the grounds that the anticholinesterase neostigmine increased chemoreflex stimulation of respiration in cats and that the effect of intracarotid injection of ACh was enhanced by anticholinesterases. These findings were confirmed by a number of authors using reflex studies (see e.g. Heymans and Neil, 1958). The introduction of electrophysiological techniques confirmed the excitatory action of ACh (Landgren, Liljestrand and Zotterman, 1952). Some authors using cats and dogs noted that this excitatory action of ACh was correlated to the nicotinic properties (e.g. Mercier, Rizzo and Delphaut, 1934; Anichkov, 1937; Philippot, 1937; Comroe and Schmidt, 1938), whereas substances that possess muscarinic rather than nicotinic properties such as the derivatives of β-methylcholine had little or no effect (Comroe and Starr, 1933; Philippot, 1937; Comroe and Schmidt, 1938). Methacholine initially considered as a muscarinic stimulant also stimulates chemoreceptors (De Wispelaere, 1937), but this effect was later clarified as inconclusive by McQueen (1978) who showed that the stimulant effect was a result of a weak nicotinic action of methacholine.

Other arguments supporting ACh as a transmitter in the carotid body include its release from the structure (Eyzaguirre, Koyano and

Taylor, 1965; Fidone, Weintraub and Stavinoha, 1976), the presence of acetylcholinesterases identified histochemically (e.g. Hollinshead and Sawyer, 1945; Koelle, 1951; Biscoe and Silver, 1966). Choline acetylase is also present in the carotid body but in small amounts (Hebb, 1968; Jones, 1975). However, some of these findings have been regarded as equivocal (see e.g. Douglas, 1954; Biscoe, 1971; Sampson, 1971; McQueen, 1977). Moreover, mecamylamine (an ACh antagonist) does not antagonize chemoreceptor excitation induced either by hypoxia or hypercapnia (McQueen, 1977) and in the rabbit ACh inhibits chemoreceptor discharge (Docherty and McQueen, 1979).

The possibility of ACh being the transmitter of an efferent synapse involving carotid sinus nerve fibres and type I cells as suggested by Neil and O'Regan (1971) and Osborne and Butler (1975) has been questioned by Goldberg, Lentz and Fitzgerald (1978) who measured the ACh content of the carotid sinus nerve and found that the amount measured was too small (0.05 ± 0.02 pmol ACh/µg protein) compared with the amounts detected in cholinergic nerves, e.g. vagus (1.03 ± 0.42 pmol ACh/µg protein). So they concluded that the chemoreceptor fibres that synapse with type I cells are not cholinergic. Furthermore, levels of ACh present in the carotid body are virtually unchanged after chronic carotid body denervation (Fidone, Weintraub and Stavinoha, 1976; Hellstrom, 1977). Kinetic studies of choline uptake by the carotid body, and the autoradiographic localization of the high affinity component of this uptake have shown similarities with other putative cholinergic systems (see e.g. Yamamura and Snyder, 1973). The chronic total denervation of the carotid body (carotid sinus and ganglioglomerular nerves cut) for 1 week to 3 months does not reduce the high-affinity component of choline uptake. This further supports the notion that

the carotid sinus nerve is not cholinergic (see Eyzaguirre and Fidone, 1980). However, it remains to be explained whether ACh is stored, in type I, type II, or in both kinds of glomus cells.

Despite these inconsistencies, interesting observations made with the use of microelectrophysiological techniques have been reported by Hayashida and Eyzaguirre (1979). These observations indirectly support the presence in the carotid body of an excitatory transmitter. They detected the presence of random electrical noise which might be the equivalent of the miniature end-plate potentials recorded from muscle fibres. According to Eyzaguirre, a main protagonist of the ACh transmitter hypothesis in the carotid body (see Eyzaguirre and Fidone, 1978) the present state of the art can be summarized as follows: "our present view of the processes of chemoreceptor excitation would suggest that stimuli affect the resting state of the glomus cells, which in turn release a 'transmitter' or 'generator' substance (possibly ACh) into the enclosed synaptic cleft to depolarize the terminals. Ending depolarization would in turn initiate the sensory discharge by acting, presumably, on the first node of the myelinated sensory fibres".

- *Catecholamines*

The presence of chromaffin tissue in the carotid body (e.g. Ross, 1959) prompted studies on the involvement of catecholamines in the carotid body. The findings supporting the importance of catecholamines are: (1) the presence of dopamine, noradrenaline and adrenaline in vesicles of the type I cells (e.g. Chiocchio, Biscardi and Tramezzani, 1966; Dearnaley, Fillenz and Woods, 1968; Zapata, Hess, Bliss and Eyzaguirre, 1969; Mills, Smith, Slotkin and Breese, 1978); (2) the enzymes required to synthetize catecholamines, specially tyrosine-hydroxylase, the rate-limiting enzyme is present in Type I

cells (Hanbauer, Lovenberg and Costa, 1977). Monoamine oxidase, which is an important enzyme in the catabolism of monoamines is also present in the carotid body (Lee and Mattenheimer, 1964); (3) the depletion of dopamine-containing vesicles by anoxia in the rat (e.g. Hanbauer and Hellstrom, 1978); (4) the presence of both α- (Sampson, Aminoff, Jaffe and Vidruk, 1976) and β- (Biscoe, 1965; Folgering, Ponte and Purves, 1980) receptors; (5) administration of dopamine close-arterial to the carotid body inhibits chemoreceptor discharge in cats (Sampson, 1972; Zapata, 1975; Docherty and McQueen, 1978) and noradrenaline and adrenaline cause small inhibition followed by an excitation (see Eyzaguirre and Fidone, 1980).

ACh and catecholamines are present in the glomus cells of the carotid body and release of these substances increases during stimulation by hypoxia or interruption of flow *in vitro*, and by pharmacologic stimuli (see Eyzaguirre and Fidone, 1980; Fidone, Gonzalez and Yoshizaki, 1980). The response of carotid body chemoreceptors to these substances varies among the mammalian species studied so far. For example, ACh increases the discharge of cat chemoreceptors through nicotinic mechanisms (Eyzaguirre and Koyano, 1965; McQueen, 1977; 1978; Monti-Bloch and Eyzaguirre, 1980) and muscarinic agonists are practically without effect. On the other hand, ACh depresses the discharge of rabbit chemoreceptors through muscarinic actions (Docherty and McQueen, 1979; Monti-Bloch and Eyzaguirre, 1980). As Eyzaguirre and Fidone (1980) pointed out recently, "it appears then that, in the rabbit, the depressant muscarinic effect of ACh predominates over the stimulatory nicotinic action of ACh". Opposite actions for dopamine have also been described, in cats and dogs the response is predominantly inhibitory whereas in rabbits both excitatory and inhibitory receptive

sites for dopamine appear to exist (see Eyzaguirre and Fidone, 1980). In rabbits *in vivo* dopamine inhibits chemoreceptor discharge (Docherty and McQueen, 1979) whereas *in vitro* it increases the discharge (Monti-Bloch and Eyzaguirre, 1980).

In spite of cholinergic and dopaminergic antagonists blocking or decreasing the effects of ACh or dopamine, they do not appreciably affect the spontaneously occurring discharge or the increase induced by natural (low PaO_2; high $PaCO_2$; low pH) or chemical (e.g. NaCN) stimuli (see e.g. Eyzaguirre and Fidone, 1980). It might be that natural stimulation releases other agents such as neuropeptides that interfere with the action of ACh and/or dopamine and, hence, make the effect of cholinergic and dopaminergic blockers less effective. Alternatively, as Eyzaguirre and Fidone (1980) suggested, one should consider the possibility of electrical coupling between glomus cells and nerve endings. These authors also consider that since ACh and catecholamines are vasoactive substances these substances can influence chemoreceptor discharge by interfering with the local carotid body blood flow.

1.5 Ouabain

Despite extensive studies on the actions of cardiac glycosides on the nervous system (for a recent review see Gillis and Quest, 1980), little is known about the effects of these substances on carotid body chemoreceptors.

Ouabain is a glycoside used in the treatment of cardiac insufficiency. A property of this group of substances is that they affect Na^+, K^+-ATPase (see e.g. Akera and Brody, 1978). The integrity of this enzymatic system seems essential for the process of normal activation

of excitable cells. This has been demonstrated in nerve and muscle (skeletal, smooth or cardiac muscle) cells. The recognition that cardiac glycosides affect the Na^+, K^+-ATPase (Schatzmann, 1953) and that via this mechanism they can trigger a number of subsequent ionic processes involved in the excitability of the cells, i.e. modify Na^+, K^+ and Ca^{2+} concentrations inside and outside the cells, has been a result of extensive studies (for reviews, see e.g. Skou, 1965; Thorp and Cobbin, 1967; Lee and Klaus, 1971; Akera and Brody, 1978; Gillis and Quest, 1980).

Transmission at the autonomic ganglia in anaesthetized cats can be enhanced by cardiac glycosides (digitoxin, lanatoside-C, K-strophantoside) and this effect is followed by an inhibition of transmission. These substances also increase the ganglionic responses to ACh (Konzett and Rothlin, 1952). Confirmation of these results was obtained by Perry and Reinert (1954) who used ouabain. Neurophysiological studies also performed *in vivo* suggest that cardiac glycosides facilitate ganglionic transmission (Pace and Gillis, 1976; Weaver, Akera and Brody, 1976). It has been shown in cats and dogs *in vivo* that high doses of cardiac glycosides increase catecholamine release from the adrenal medulla (Richards and Wood, 1915). This has been confirmed *in vitro* by Banks (1967) who reported that ouabain increases both spontaneous and evoked (carbachol-induced) release of catecholamines by the isolated perfused adrenal gland of the cow, and by others using adrenals from dogs, cats and rats (Roy and Chatterjee, 1970; Gaseon, 1977; Garcia, Hernandez, Horga and Sanchez-Garcia, 1980). It has been frequently suggested that the excitatory effect of cardiac glycosides in neural tissue is related to their action on the Na^+, K^+-ATPase (see e.g. Gillis and Quest, 1980).

The presence of this ATPase has been identified in small nerve fibres (Rang and Ritchie, 1968). Thus inhibition of this ATPase by cardiac glycosides could explain the excitatory action in efferent autonomic nerves (Ten Eick and Hoffman, 1969) and ganglia (Libet, Tanaka and Tosaka, 1977; Smith and Weight, 1977). Myelinated fibres such as motor nerve fibres are also excited by cardiac glycosides either *in vivo* (Levitt, Raines, Sohn, Standaert and Hirshfeld, 1970) or *in vitro* (Birks, 1963) and the glycosides also stimulate these fibres, probably by inhibition of the sodium pump.

There is evidence that chemosensory receptors in the region of the carotid bifurcation can be affected by cardiac glycosides. Schmitt, Guth and Müller-Limmroth (1958) were the first to show that digitalis can act on carotid body chemoreceptors. They described that as a consequence of injecting digitalis (digipurat and K-strophanthin) intravenously in cats the frequency of action potentials recorded from chemoreceptor fibres increases. Their identification of chemoreceptors was based on the localization of the recording electrodes in the carotid body, and that increasing PCO_2 in the ventilating air increased spontaneous chemoreceptor discharge. More recently, McLain (1970) described how ouabain injections (i.m. or i.v.) in cats anaesthetized with chloralose increase neural traffic (baroreceptor and chemoreceptor activity) recorded from the carotid sinus nerve, and Joels and Neil (1968) reported that ouabain (10^{-10}M) inhibits chemoreceptor activity evoked by nitrogenated Krebs-Hensleit solution and in a concentration of 10^{-8} M can also reduce the discharge evoked by ACh in vascularly isolated carotid bodies. According to these authors the effect of ouabain on ACh responses might suggest that ouabain has some action on the chemosensory endings. Studies performed *in vitro* by Eyzaguirre,

Baron and Gallego (1977) have shown that ouabain (5×10^{-5}M) induces cell depolarization of the type I cells at normal temperature and this effect was potentiated by lowering the temperature of the perfusion fluid. More indirect observations also indicate that cardiotonic glycosides can activate chemoreceptors. For example, Heymans, Bouckaert and Regniers (1932) and Zipf and Ehrlicher (1951) reported that digitalis can activate carotid sinus receptors. However, it is not possible to ascertain whether these effects were exerted on baroreceptors, chemoreceptors or both, since selective discrimination between the different sensory receptors was not attempted. It has been found that in decerebrate cats in which the carotid bodies were destroyed by acetic acid, intracarotid acetylstrophanthidin caused a smaller effect on carotid sinus nerve activity as compared with the responses obtained before destruction (Quest and Gillis, 1971). Another glycoside, scillaren A injected close-arterial to the carotid body in dogs enhanced the reflex respiratory excitatory responses to ACh and nicotine (Carpi, Konzett and Cerletti, 1957). In an investigation performed by Viana (1973) digoxin (2 µg/kg/min i.v.) potentiated the respiratory stimulant effects of NaCN (i.c.) and ACh (i.c.). Furthermore, digoxin (2 µg/kg/min i.v.) no longer caused respiratory stimulation after surgically removing both carotid bodies in dogs (Viana, 1974). The relative contribution of chemoreceptors as opposed to baroreceptors cannot be ascertained from these studies.

In the present thesis the effects of ouabain on arterial chemoreceptors have been studied in order to clarify its action on spontaneous chemoreceptor discharge and its interaction with evoked responses.

1.6 Adenosine triphosphate

It is well-known that the chemical, mechanical and osmotic work of the cells depends upon the chemical energy supplied by ATP (see e.g. Lehninger, 1965). According to this, therefore, ATP should affect the carotid body, and a theory for the activation of the carotid body based on the interruption of its ATP production has been developed. This theory has been advanced on the grounds that anoxia and metabolic inhibitors increase chemoreceptor discharge (see e.g. Krylov and Anichkov, 1968; Torrance, 1968). The action of anoxia or of metabolic inhibitors on cells is to interfere with the energy supply to the Na^+ pump (Hodgkin and Keynes, 1955) and to the Ca^{2+} pump (for a review see e.g. Carafoli and Crompton, 1978). All this information comes from indirect evidence. As Loewenstein and Rose (1978) pointed out, it is possible to cause closure of Ca^{2+} channel in condition in which the cells are poisoned with cyanide, but there is no way of knowing whether in this condition all ATP was actually depleted. ATP can restore and enhance the responsiveness of the carotid body when this has been reduced by prolonged perfusion with poisons (e.g. cyanide) (see Anichkov and Belen'Kii, 1963). Also Joels and Neil (1968) found that in vascularly isolated perfused cat carotid body ATP reduces the responses to cyanide or dinitrophenol, which seems to support the ATP depletion theory. The results of the Russian school seem to imply that ATP exogenously applied can restore chemoreceptor activity when it is exhausted, whereas Joels and Neil (1968) suggest that ATP protects against metabolic inhibitors. Although subscribing the same theory, i.e. relying on ATP depletion for chemoreceptor activation, their results come from different observations. The Russian school reported that the nucleotide is ineffective in low concentrations but in high

concentrations excites chemoreceptors, whereas Joels and Neil (1968) found that ATP (10^{-5} w/v) reduced chemoreceptor responses evoked by cyanide or dinitrophenol; this implies that chemoreceptor activity is reduced during normal production of ATP. AMP and ADP lacked the effects of ATP (Joels and Neil, 1968). Although the observations of these authors fit well the ATP-metabolic chemoreceptor activation, their results appear to be rather a matter of coincidence than to serve as real support to the theory, since (1) ATP hardly crosses cell membranes from outside to inside the cells (see e.g. Glynn, 1968), and this is theoretically supported by knowing that the normal concentration of ATP inside the cells (1-5 mM) is much higher than outside (see Lehninger, 1965); (2) the enzymatic machinery quickly metabolizes ATP into ADP, AMP, adenosine, adenine, inosine and hypoxanthine. Roumy and Leitner (1977) have been able to reproduce the results of Krylov and Anichkov (1968) but not those reported by Joels and Neil (1968); they proposed that calcium is involved in the process of chemoreceptor activation on the grounds, firstly that transmitter release from the type I cells during hypoxia is a function of the cytoplasmic calcium concentration, and secondly that the concentration of cytoplasmic calcium is regulated by the mitochondria which has an ATP-dependent calcium pump (see e.g. Carafoli and Crompton, 1978). Similar interpretations have been used to explain the increased release of transmitter at the neuromuscular junction in the presence of metabolic inhibitors (see e.g. Alnaes and Rahaminoff, 1975) as well as to explain the decrease in the spontaneous release of the transmitter at the neuromuscular junction caused by both adenosine and ATP (Ribeiro and Dominguez, 1978). So when less ATP is present in the cell more calcium is free in the cytoplasm to promote transmitter release, because the

mitochondrial ATPase (the calcium pump) cannot pump calcium into the mitochondria in the absence of ATP. Mimicking the intracellular ATP by using exogenous ATP appears, therefore, difficult to achieve, but even if it were possible to do this the ATP would be expected to decrease rather than increase chemoreceptor activity. The analysis of the above information seems to indicate that ATP activates chemoreceptors via a site located outside the membrane. A theory concerning the role of ATP in the process of impulse generation has been proposed by Duncan (1965, 1967). According to this, the mechanism underlying the generator process at sensory nerve endings is similar to that used by amoeba in sensing mechanical stimuli, i.e. there is an enzyme (ATPase) located on the membrane which contacts with the substrate and triggers the activation (Duncan, 1967).

Despite the discovery that ATP is the main energy supplier for the work of the cells, which created conditions to advance metabolic theories of nerve activation and release of transmitters, ATP, related nucleotides and adenosine have been progressively implicated in neurotransmission quite independently of their direct metabolic roles as part of the cellular energy supply system.

It is nearly 30 years since Holton and Holton (1953, 1954) suggested that ATP might be released as a neurotransmitter from both peripheral and central endings of sensory fibres. Since then a great deal of effort has gone into attempts to unravel the role of adenosine and adenine nucleotides on neurotransmission. Torrance (1968) pointed out the interest of the excitatory action of ATP reported by Anichkov and Belen'Kii (1963) in view of the report of Douglas and Poisner (1966) that ATP is released with catecholamines from the adrenal medulla and since catecholamines have their supporters for the office of transmitter

in the carotid body, ATP may act synergically (cotransmitter function) with an amine at the nerve endings.

The actions of adenosine and related nucleotides on neurotransmission have been a matter of intensive research in the last ten years (for reviews see e.g. Burnstock, 1972; McIlwain, 1972; Arch and Newsholme, 1978; Ribeiro, 1978; Fredholm and Hedqvist, 1980; Daly, Bruns and Snyder, 1981; Phillis and Wu, 1981; Stone, 1981).

There are in principle, two distinct actions of adenosine and related nucleotides at synapses. One is the postsynaptic action - ATP can behave as a neurotransmitter and/or as a cotransmitter. The role of ATP as a neurotransmitter has been extensively characterized by Burnstock (1972, 1978) under the concept of purinergic transmission. For a recent account of the steps which led to the formulation of the purinergic hypothesis, the recent monograph Purinergic Receptors (Burnstock, 1981) should be consulted.

The function of ATP and/or adenosine as cotransmitters is poorly understood, but basically corresponds to their capacity to sensitize the post-synaptic membrane to the effects of the classical neurotransmitters such as ACh or noradrenaline (see e.g. Ribeiro, 1978).

The other action of adenosine and related nucleotides at synapses is presynaptic and manifest by a reduction in transmitter release. This was first detected electrophysiologically by Ginsborg and Hirst (1971; 1972) in the rat-diaphragm, and further confirmed in the same laboratory by Ribeiro and Walker (1973; 1975) in both rat-diaphragm and the frog-sartorius preparations. There is now an extensive literature on the presynaptic action of adenosine and adenine nucleotides (e.g. Ribeiro, 1979; Burnstock, 1980; Fredholm and Hedqvist, 1980; Paton, 1981; Stone, 1981).

In spite of these achievements more direct evidence for the presence of ATP in the carotid body was produced in 1972 by Nada and Ulano, who detected histochemically and by electron microscopy adenosine triphosphatase activity in the carotid body. However, its localization to vesicles and, hence in a releasable state, was only recently reported by Böck (1980). This author used fluorescence microscopy (labelling with quinacrine) and electron microscopy (uranaffin reaction) and concluded that ATP is stored within specific granules in addition to catecholamines and proteins in the carotid body type I cells. Part of his work was prompted by (1) previous descriptions concerning the storage mechanisms of catecholamines in dense-core vesicles of sympathetic terminals and in adrenomedullary chromaffin granules (Smith and Winkler, 1972); and (2) by the prediction that paraneurones (Fujita and Kobayashi, 1979) should contain amines, peptides and ATP.

ATP is effective on sensory systems. For example, perfusion of frog taste buds, via the lingual artery, with ATP initiates a discharge in the afferent nerve (Duncan, 1964); ATP excites sensory nerve endings of trigeminal (Juan and Lembeck, 1974). Adenosine compounds excite pain receptors (Bleehen and Keele, 1977) and their action on afferent nerve terminals resemble other known algogenic substances such as potassium, ACh, 5-HT and bradykinin. The adenine compounds were comparatively less potent than ACh, 5-HT or bradykinin, but had greater potency than potassium (Bleehen, 1978).

In the perfused superior cervical ganglion of the cat phosphate compounds such as ATP stimulate ganglionic transmission (Feldberg and Hebb, 1948). In isolated spinal cord preparations in which synaptic transmission had been abolished by perfusing with a Ca^{2+}-free solution

containing 10 mM Mg^{2+}, ATP had a depolarizing action on dorsal root terminals but did not affect motoneuronal membrane potentials. It is likely that these compounds have a direct effect on afferent terminals (Phillis, Kastopoulos, Edstrom and Ellis, 1979).

Of more direct interest to the present work are the observations of Jarisch, Landgren, Neil and Zotterman (1952) who found that ATP increases chemoreceptor discharge when injected i.c. The effect was interpreted as a result of stimulating the sensory nerve endings, and compared to that observed by Jarisch and Zotterman (1948) who demonstrated that ATP can stimulate cardiac receptors, and is also comparable with the stimulation of pulmonary nerve endings (Emmelin and Feldberg, 1948). Dontas (1955) also found that ATP injected i.c. causes chemoreceptor excitation, and that the effect is probably on the membrane surface. Furthermore, he concluded that inactivation of phosphorylation should not be responsible for chemoexcitation, since some inhibitors such as thiopentone and pentobarbitone depressed chemoreceptor activity.

Some of the above authors concentrated on studying the effects of ATP but concluded that ADP and AMP were also effective, although only in much higher doses. So far there have been no reports on the effects of adenosine.

The first hint that adenosine modifies physiological processes came in 1929 when Drury and Szent-Gyorgi observed that injection of adenosine into mammals lowered the arterial blood pressure, dilated the coronary arterioles, induced sleep and inhibited movements of the small intestine.

As Arch and Newsholme (1978) pointed out, during the thirty years that followed the observations of Drury and Szent-Gyorgi (1929),

more attention was given to the pharmacological effects of adenine nucleotides than those of adenosine. In particular, attention was centred on ATP. Upon injection, high doses of ATP produce a state of shock with lowered blood pressure, lowered body temperature, renal dysfunction, mobilization of glycogen from liver and muscle and elevated blood levels of glucose, lactate and pyruvate. This suggested that the release of ATP from damaged tissues contributed to traumatic shock (Green and Stoner, 1950). Although adenosine produced similar effects to ATP, as Arch and Newsholme (1978) pointed out, the tissue content of adenosine was considerably less than that of ATP so that it was considered to be less important. According to Arch and Newsholme (1978) if the rapidity of the breakdown of extracellular ATP to adenosine had been appreciated, the nucleoside might have assumed a greater importance in these early studies.

ATP is present in the carotid body type I cells but there is conflicting evidence regarding its action on the chemoreceptors. Recent ideas about the importance of other purines, in particular adenosine, on neurotransmission led to the present investigation concerning the effects of these substances on arterial chemoreceptors.

1.7 Peptides

As a result of recent progress in understanding the nervous system a number of neuropeptides emerged as fulfilling a number of criteria classically required of neurotransmitters namely on synthesis, storage, release, inactivation in tissues and effectiveness when exogenously applied (for reviews see e.g. Lord, Waterfield, Hughes and Kosterlitz, 1977; Iversen, 1979; Hökfelt, Johansson, Ljungdahl, Lundberg and Schultzberg, 1980; Snyder, 1980). Their importance

has been stressed in terms of their ambivalence, i.e. being either neurotransmitters, hormones or having both roles according to the circumstances. As mentioned previously ideological support for these roles has been provided by the APUD and paraneurone concepts.

Using immunohistochemical techniques such as the indirect immunofluorescence technique of Coons (1958) (Lundberg, Hökfelt, Fahrenkrug, Nilsson and Terenius, 1979; Cuello and McQueen, 1980; Fitzgerald, Raff, Garger, Fechter, Anand and Said, 1981) or combining immunohistochemistry and radioimmunoassay (Wharton, Polak, Pearse, McGregor, Bryant, Bloom, Emson, Bisgard and Will, 1980) it has proved possible to distinguish enkephalin-, substance P- and VIP-like immunoreactivity in the carotid body.

As Hökfelt, Johansson, Ljungdahl, Lundberg and Schultzberg (1980) pointed out any substance can be traced using immunohistochemistry providing that (1) the substance is available in pure form, (2) it is immunogenic (or can be rendered immunogenic by conjugation to carrier protein) and (3) it can be retained in tissue sections during processing for immunohistochemistry.

Apart from limitations of sensitivity, and the limited ability of the antibodies to penetrate the intracellular storage sites, the major problem is the specificity of the immunological reaction. Thus, an antiserum may not only react with the proper antigen but also cross-react with structurally similar peptides.

Important characteristics of peptides are that they can consist of up to 30 or more amino acids and high molecular weight (M.W. $\simeq$ 3000) compared with the classical neurotransmitters (M.W. $\simeq$ 200).

One feature that is common to several classes of peptide neurone is the large granular vesicle (Goldsmith, 1977) compared with 'pure'

cholinergic and adrenergic neurones containing small granular vesicles (see Burnstock, Hökfelt, Gershon, Iversen, Kosterlitz and Szurszewski, 1979). The method of replenishment of peptide neurotransmitter at nerve endings seems to differ from that of classical neurotransmitters. For example, intraneuronal noradrenaline levels are kept fairly constant by the efficient replacement of released transmitter by (1) enzymatic synthesis in the nerve endings, (2) re-uptake from the extraneuronal (synaptic) space through an active membrane mechanism and (3) supply of amine in storage vesicles (or their precursors) from the cell body via axonal transport (Iversen, 1967).

Peptides, on the other hand, are probably produced only on the ribosomes of the cell soma, possibly in the form of a larger precursor molecule (Gainer, Loh and Sarne, 1977) without local synthesis in nerve endings. As no reuptake mechanisms seem to operate for peptides in nerve endings, every single peptide molecule released from a nerve ending must be replaced by axonal transport. This comparatively inefficient and slow mechanism should be reflected in the dynamics of synaptic events at peptide synapses (Bloom, 1977).

Table 1.1 summarizes main similarities and differences between classical small neurotransmitters and putative peptide transmitters that should be kept in mind, according to Hökfelt (1979), in order to understand potential mechanisms of action involved in the effects of both groups of substances.

1.7.1 Opioid peptides: methionine-, leucine-enkephalin and β-endorphin

Hughes (1975) showed that brain extracts can mimic morphine's effects on electrically induced contractions of smooth muscle and that this is blocked by naloxone. A morphine-like substance from the brain of pigs was isolated and shown to consist of two pentapeptides,

TABLE 1.1: Differences between classical small neurotransmitters and putative peptide transmitters (Hökfelt, 1979)

	Classical transmitters	Putative peptide transmitters
Molecular weight	≃ 200	≃ 3000[1]
Synthesis		
Principle	Enzymatic	Ribosomal
Localization	Nerve endings (cell body, axon)	Cell body
Storage	Vesicles	Vesicles
Supply for release	Local synthesis Reuptake (Axonal transport)	Axonal transport Cleavage from precursor
Inactivation	Reuptake Enzymatic Diffusion	Enzymatic Diffusion

[1] Exceptions are the enkephalins with molecular weights about 600.

met-ENK and leu-ENK (see Figure 1.2) which differ only in having methionine or leucine at the carboxyl terminal. The same two peptides were also isolated from calf brain (Simantov and Snyder, 1976). By using radioreceptor assay it was shown that the enkephalins were localized in nerve endings (Simantov, Snowman and Snyder, 1976), this being consistent with a neurotransmitter role, and in the monkey brain their regional distribution closely parallels that of opiate receptors. Moreover, it was described that the enkephalins are released in a calcium-dependent manner with brain depolarization (e.g. Smith, Hughes, Kosterlitz and Sosa, 1976; Iversen, Iversen, Bloom, Vargo and Guillemin, 1978).

The five amino acids (residues) constituting met-ENK are contained within the 91 amino acids of the peptide β-lipotropin isolated from the pituitary by Li (1964). Several groups of investigators showed that a variety of lipotropin fragments, all incorporating the met-ENK possess opiate activity (e.g. Ling, Burgus and Guillemin, 1976). Within the pituitary most opiate-like activity can be accounted for by β-endorphin, the 31 amino acid peptide at the carboxyl terminal portion of β-lipotropin (Goldstein, Tachibana, Lowney, Hunkapiller and Hood, 1979). β-lipotropin itself derives from a 31,000 dalton precursor peptide which also incorporates the sequence of ACTH, and hence is referred to as 31K ACTH (e.g. Mains, Eipper and Ling, 1977). β-endorphin occurs in the brain in specific neuronal systems (e.g. Rossier, Vargo, Minick, Ling, Bloom and Guillemin, 1977).

As Hughes (1978) pointed out recently, β-endorphin may be considered as the prototype opioid peptide interacting equally well with several or perhaps all opiate receptors. Met- and leu-ENK are more sensitive to rapid proteolytic inactivation and have more

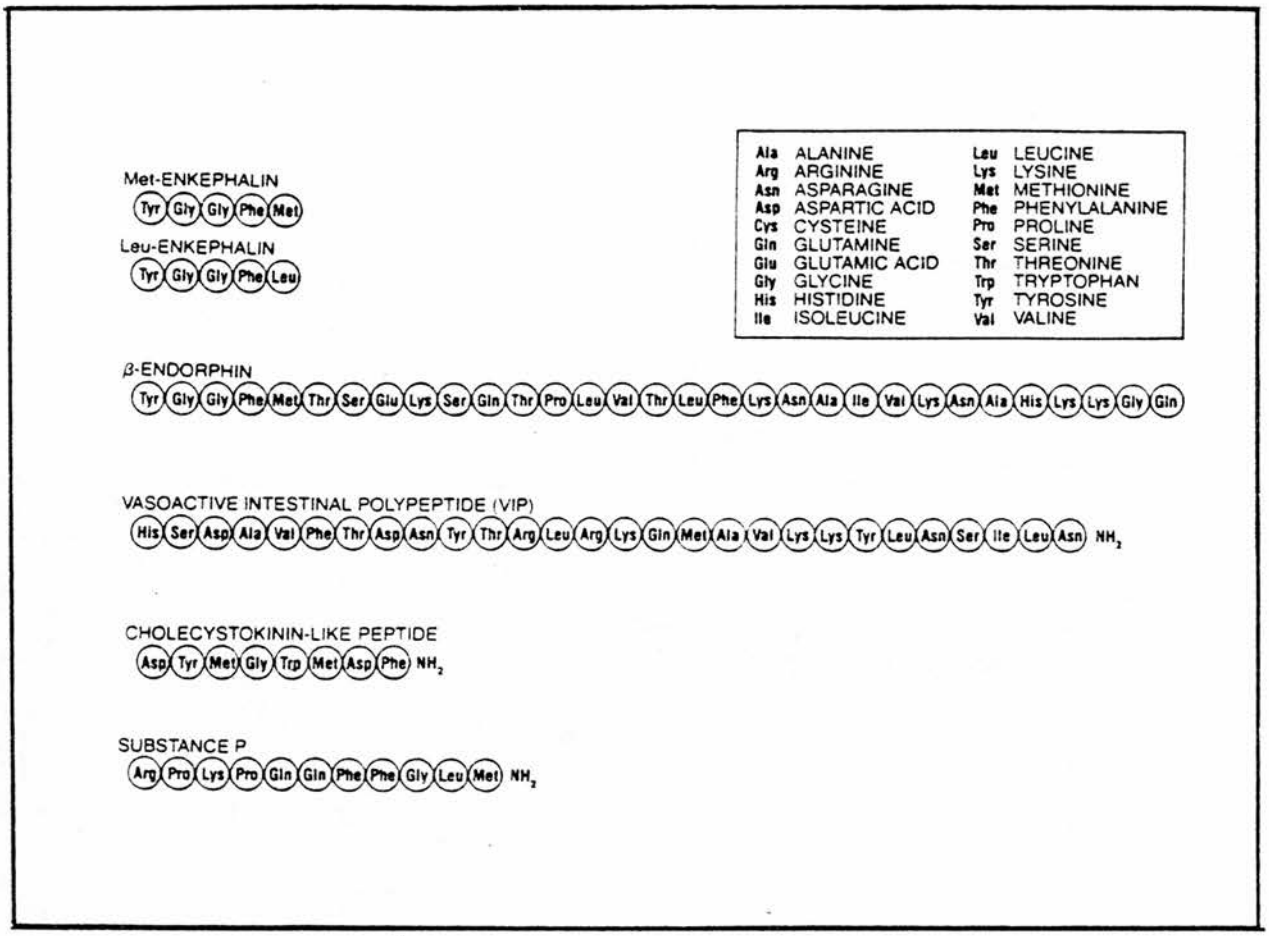


FIGURE 1.2

Composition of the neuropeptides studied in the present work.

specific receptors. According to these authors, β-endorphin should be considered as having mainly a neuroendocrine function whereas the enkephalins may have a neurotransmitter function.

Vasoactive intestinal polypeptide

VIP was identified in extracts of the gut as a vasodilator. It was isolated as a 28 amino acid peptide (see Figure 1.2) with many similarities in amino acid sequence and biological activity to the intestinal peptides secretin and glucagon, namely stimulates the conversion of glycogen to glucose, increases lypolysis and insulin secretion, decreases the production of gastric acid, and stimulates secretion by the pancreas and small intestine (Said and Mutt, 1970; Mutt and Said, 1974). VIP was also found in the brain, the highest levels occur in the cerebral cortex (Bryant, Polak, Modlin, Bloom, Albuquerque and Pearse, 1976; Said and Rosenberg, 1976). The VIP neurones are bipolar and oriented perpendicular to the surface of the cerebral cortex. This makes them ideally suited to activate and synchronize neuronal activity within the vertical columns of cerebral cortical cells. VIP is a potent and rapid neuronal excitant in the hippocampus, is stored in vesicles and is released with neuronal depolarization (see Snyder, 1980).

It is interesting to note that the concentrations of VIP in the carotid bodies and in the carotid sinus nerves from the cat are relatively close (carotid body/carotid sinus nerve = 1.46). This contrasts with the striking difference in relation to ACh (carotid body/carotid sinus nerve = 3-4 or 30-40 depending on how ACh is determined - see above). VIP infused close arterial to the carotid body increases chemoreceptor activity (Fitzgerald, Raff, Garger, Fechter, Anand and Said, 1981). This is statistically significantly different from the control at 15 and 20 s

intervals. The doses of 5 or 25 μg in 0.5 ml in six experiments each caused similar effects, i.e. about 50% increase in relation to the pre-injection period. Arterial pressure dropped in a dose-dependent manner with the diastolic pressure being most affected, a characteristic response to VIP.

Cholecystokinin octapeptide

CCK was originally isolated from the duodenum as a substance that contracted the gall bladder. It is a 33 amino acid peptide (Mutt and Jorpes, 1968) and is identical to pancreozymin. Whereas intestinal CCK largely consists of the 33 amino acid residue peptide, the major CCK entity in the brain is the COOH-terminal octapeptide (CCK-8) (see Figure 1.2) with a lesser amount of the COOH-terminal tetrapeptide and very little CCK-33 (Muller, Straus and Yalow, 1977; Rehfeld, 1978).

CCK is present in the cerebral cortex and with VIP are the only brain peptides with cells in the cerebral cortex (see Snyder, 1980). The concentration of CCK in total brain is about 1-2 mg in humans, far greater than that of any other peptide (see Snyder, 1980). CCK-8 is a quick and potent excitant of cerebral cortical cell firing with a fast onset than ACh (Dodd and Kelly, 1979).

Like SP, CCK occurs in sensory fibres with cell bodies in dorsal root ganglia and terminals in the dorsal grey matter of the spinal cord. As with SP, neurotensin and the enkephalins CCK cells are abundant in the hypothalamus, whereas the central nucleus of the amygdala has a dense collection of CCK fibres but not cells (see Snyder, 1980). Like VIP neurones CCK neurones are positioned in a similar way in the cerebral cortex and hence similar function has been postulated for both substances at this level (Snyder, 1980). So far no CCK-8 has been detected in the carotid body.

Substance P

Next to the enkephalins, SP (see Figure 1.2) is the most studied brain peptide. The role of SP as a sensory transmitter in the pain reflex pathway has been extensively supported. It occurs in 20% of dorsal root ganglia cells with some processes extending to the skin and others entering the spinal cord and giving rise to terminals in the substantia gelatinosa (see Hökfelt, Johansson, Ljungdahl, Lundberg and Schultzberg, 1980). SP has been associated with sensory nerves. For example, removal of the tooth pulp, which contains only pain-sensitive sensory fibres, causes a loss of nerve endings that contain SP in the trigeminal nucleus of the brainstem, where sensory fibres terminate (Gobel and Binck, 1977).

Since SP is a 'pain' transmitter, the demonstrated blockade of its release in spinal cord by opiates (Jessel and Iversen, 1977; Mudge, Leeman and Fishbach, 1979) may account in part for opiate analgesia. Interactions between the enkephalins and SP may occur in the central nervous system, since neurones containing the two peptides are juxtaposed in such areas as the raphe nuclei, the ventral tegmental area, the septum and the amygdala (see Hökfelt, Johansson, Ljungdahl, Lundberg and Schultzberg, 1980).

The effects of SP on carotid chemoreceptor activity in the cat have been studied by McQueen (1980) who found that SP causes a delayed increase in spontaneous chemoreceptor activity.

The coexistence of peptides with classical transmitters and ATP is now largely accepted (see e.g. the Neurosciences Research Program edited by Burnstock, Hökfelt, Gershon, Iversen, Kosterlitz and Szurszewski, 1979). In many tissues coexistence of receptors for classical neurotransmitters such as ACh and catecholamines, for peptides

such as the opioid peptides (met-, leu-ENK and β-endorphin) and for ATP and/or its metabolite adenosine, has been reported. Co-existence of substances has been found in the adrenal medullary cells (Schultzberg, Lundberg, Hökfelt, Terenius, Brandt, Elde and Goldstein, 1978) and in the carotid body (Lundberg, Hökfelt, Fahrenkrug, Nilsson and Terenius, 1979). The neurones that innervate exocrine glands, including sweat and salivary glands and glands in the nasal mucosa and tongue are innervated by cholinergic neurones, which contain a VIP-like peptide (Lundberg, Hökfelt, Schultzberg, Uvnäs-Wallenstein, Kohler and Said, 1979). This is based on techniques that identified in the same sections the existence of ACh-esterase rich neurones containing VIP. This suggests that these neurones may release two putative transmitters, ACh and VIP (Fahrenkrug, Haglund, Jodal, Lundgren, Olbe and Schaffalitzky de Muckadell, 1978). For instance, it has been postulated that ACh and VIP can be released from the same nerve endings to participate in the secretory process: ACh mainly causing secretion and VIP vasodilatation (see Hökfelt, Johansson, Ljungdahl, Lundberg and Schultzberg, 1980). The VIP response is atropine-resistant (e.g. Ängaård, 1974). In the salivary gland simultaneous infusion of VIP and ACh causes a marked potentiation of both vasodilatary and secretory responses (Lundberg, Ängaård, Fahrenkrug, Hökfelt and Mutt, 1980).

The presence of neuropeptides in the carotid body together with their apparent importance in neurotransmission in other parts of the nervous system prompted the present interest on the investigation of their effects on carotid body chemoreceptors.

SECTION II

Methods and Materials

Experiments were performed on 71 cats of either sex (26 females and 45 males) weighing between 1.7 and 4.4 kg (mean 3.03 ± 0.05 kg). In 2 cats no data were obtained: from the other 69 cats, 21 recordings were from single chemoreceptor units and 48 from multiple units.

Anaesthesia

The animals were anaesthetized with pentobarbitone sodium, 42 mg/kg i.p., supplemented approximately every 1-2 hr during the experiment by 10% of the initial dose administered i.v.

General

A cannula was inserted into the trachea low in the neck. Both femoral arteries were cannulated, one catheter being connected to a B.P. transducer (Bell and Howell, 4-442) and the other used for withdrawing blood samples for gas analysis. The signal from the pressure transducer was displayed on a pen-recorder (Ectromed, Mx 6) and recorded on one channel of an FM tape recorder (Tandberg, 100; frequency response d.c. to 1250 Hz). Arterial blood pH, PO_2 and PCO_2 were measured at hourly intervals using a Radiometer gas monitor (BMS 3 with PHM 71 meter). A femoral vein was cannulated and used for drug administration. Rectal temperature was monitored and maintained at 38 ± 0.5°C by an electric blanket.

The carotid bifurcation region was exposed and dissected free of surrounding tissue. A cannula was inserted into the lingual artery until its tip lay in the common carotid artery 1.5-2.0 cm caudal to the carotid bifurcation. In some experiments a second catheter was positioned in the same common carotid artery via the superior thyroid artery about 3 cm caudal to the tip of the lingual catheter. The

catheter dead-space was 0.3 ml. In the experiments in which recordings were made from both carotid sinus nerves another catheter was similarly positioned in the other common carotid artery. The catheters were used for both injections (0.1 ml) or infusions of drug solutions (0.1-0.5 ml/min using a Unita pump, Braun).

Respiration

The animals were artificially ventilated with room air by a respiratory pump (S.R.I.), operating at 18 rev./min. End-tidal CO_2 was continuously monitored by an infra-red CO_2 analyser (med 1A; Grubb Parsons) and maintained at about 5% by appropriate adjustment of the pump stroke volume. Hypoxic stimulation was achieved by ventilating the animal with 10-15% O_2 : 90-85% N_2 or 100% N_2. Adequate proportion of the ventilating gases was achieved with flow meters.

Gallamine

The animals were paralysed during the experiment with gallamine triethiodide (3 mg/kg i.v.), the dose being repeated as required, usually every 1 - 1.5 hr, when supplementing the anaesthesia. This neuromuscular blocking drug was given to prevent muscle contractions, either spontaneous or caused by the close-arterial injections of ACh, from moving the nerve on the recording electrodes, and also to suppress spontaneous respiratory movements which are associated with fluctuations in end-tidal CO_2 and B.P. According to McQueen (1977) chemoreceptor discharge obtained with the animal artificially ventilated and paralysed is very similar to that observed when it was breathing spontaneously, and remains relatively constant throughout the experiment. Gallamine does not appreciably affect chemoreceptor activity evoked by NaCN or ACh (see McQueen, 1977).

Recording of sinus nerve activity

The sinus nerve was identified and sectioned central to its junction with the glossopharyngeal nerve. Exposed tissues were covered with warm (37°C) mineral oil. The outer sheath of the nerve was removed and small filaments were dissected from the peripheral end of the nerve trunk. Electrical activity from single or multiple chemoreceptor units was recorded from the filaments using bipolar platinum-iridium electrodes. Sensory nerve discharge was amplified by an A.C. amplifier (Neurolog, Digitimer), displayed on an oscilloscope (Tektronix 5103N) and recorded on one channel of the tape recorder (see Figure 2.1).

Chemoreceptor units were identified by their random discharge, their increase in discharge frequency following injection of NaCN (usually 5 μg) into the ipsilateral common carotid artery, their increase in discharge in response to hypoxia, and by the depression of spontaneous discharge in response to hyperoxia (breathing 100% O_2).

In the majority of the experiments, the ganglioglomerular nerves (two to three sympathetic nerves from the superior cervical ganglion to the carotid sinus region) were cut in order to eliminate reflex effects of sympathetic activity on chemosensory discharge (Floyd and Neil, 1952; Eyzaguirre and Lewin, 1961).

Single-chemoreceptor units were identified from the constant shape and amplitude of the action potential, and by delay of at least 7 ms between successive spikes (Biscoe and Taylor, 1963). In most of the multi-unit recordings it was found that a high dose of stimulant evoked a maximum discharge which remained fairly constant during the course of an experiment. If a unit was recruited, or another ceased responding, the maximum discharge changed and this provided a quick method for

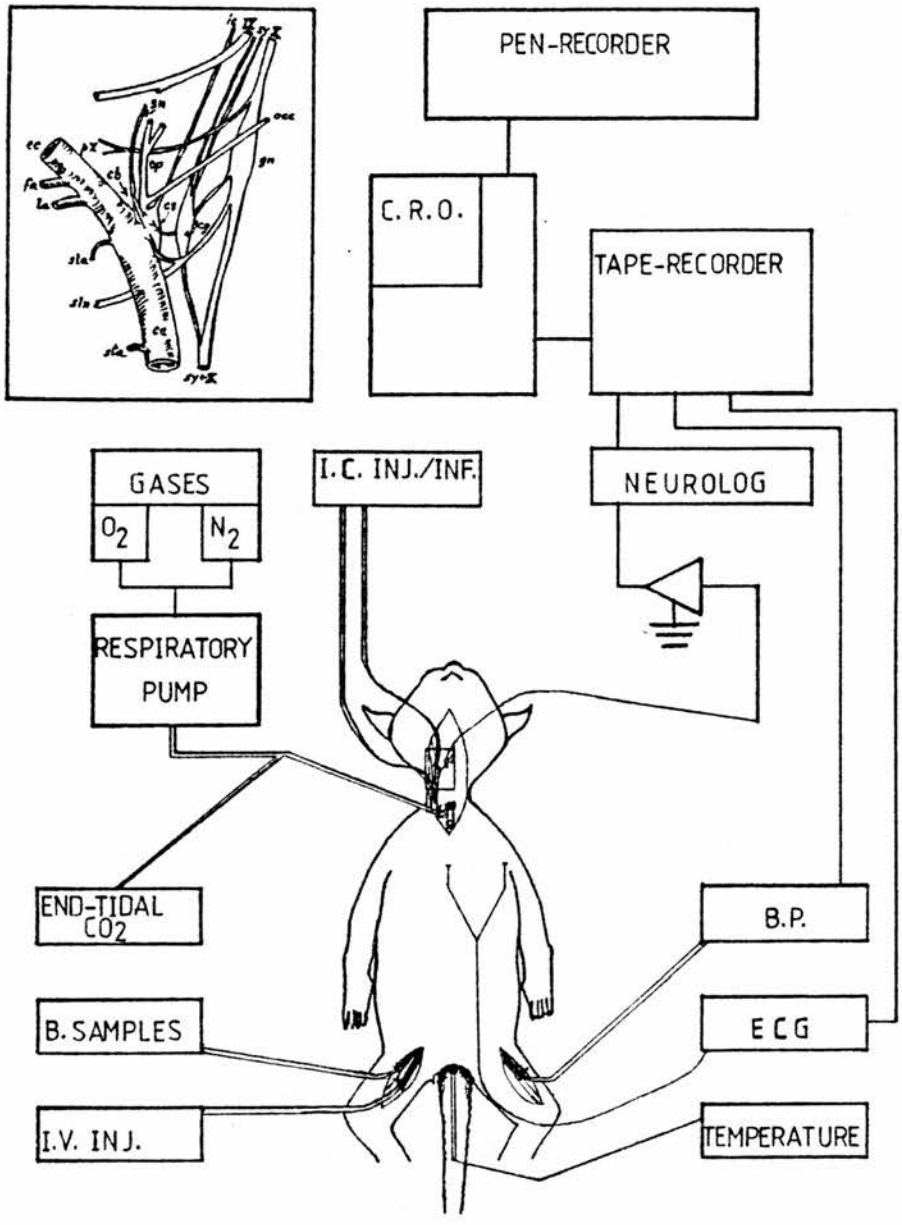


FIGURE 2.1

Schematic representation of the recording, monitoring and drug administering systems. The monitoring system includes: rectal temperature; electrocardiogram (ECG); femoral arterial blood pressure (B.P.); femoral arterial blood samples for determining pH, PaO_2, $PaCO_2$ (B.SAMPLES); end-tidal CO_2. The recording system includes a headstage, A.C. pre-amplifier, A.C. amplifier, an audio-amplifier and a tape-recorder. The drug administering system includes: intracarotid injections or infusions through catheters inserted via the lingual or superior thyroid arteries (I.C. INJ./INF.) and intravenous injections (I.V. INJ.) via a femoral vein. Different gas mixtures could be administered through the respiratory pump.

In the upper left of the figure the anatomy of the region (right side) is represented. The carotid sinus nerve (sn) from which the recordings were obtained can be seen, as can the relative positions of the lingual (la) and superior thyroid (sta) arteries in relation to the carotid body (cb). The figure also shows the ganglioglomerular (sympathetic) nerves which course between the superior cervical ganglion (scg) and the carotid body.

monitoring units and was used in conjunction with visual checking of the action potentials on the oscilloscope (see McQueen, 1977). Analysis was performed only if the number of units being examined, usually 2 - 3, remained constant throughout the experiment.

Drug administration

Intracarotid injections

Intracarotid injections were generally made in a volume of 0.1 ml into the common carotid artery and washed in with 0.2 ml Locke modified solution which had been bubbled with 5% CO_2 : 95% air in a water bath at 37°C. Injections were made at the peak of the inspiratory phase of the respiratory cycle and generally completed within one respiratory cycle, usually within 2 s. A period of 3 - 5 min was allowed between injections of test drugs, unless otherwise indicated in the text. Drugs were injected intravenously in a volume of 0.2 - 1.0 ml and the catheter flushed with 0.5 ml 0.9% w/v aqueous sodium chloride (saline). Responses of the chemoreceptors to CO_2 were obtained by i.c. injection of 0.1 - 0.3 ml Locke solution equilibrated with 100% CO_2.

Control injections of modified Locke, i.e. 0.1 ml Locke equilibrated with room air, were administered i.c. and the catheter flushed with 0.2 ml Locke bubbled with 5% CO_2 in air, in every experiment.

Data analysis

Data were analysed according to McQueen (1977). The output of the tape channel containing the action potentials passed through a filter module (DC - 10 KHz with 50 Hz notch) and fed to a pulse height discriminator (WPI mod. 120) and information relating to the number of pulses occurring in 0.1 s bins was processed by a PET 32K microcomputer

(Commodore, 303 series). The data were stored on a dual drive floppy disk (3040 series) and subsequently analysed with the aid of the microcomputer (see Figure 2.2).

Average discharge ($\bar{x}$) in the pre-stimulus (injection or infusion) or 'control' period, generally 20 - 50 s, unless otherwise indicated in the text, was computed from data stored on the floppy disk. The average and total counts (Σx) were calculated for each response after its duration (t s) had been determined by the computer. Responses were expressed as increments above the control level by subtracting the appropriate values, i.e.:

$$\Delta\bar{x} = \bar{x}\text{ (response)} - \bar{x}\text{ (control)}$$
$$\Delta\Sigma x = \Sigma x\text{ (response)} - \Sigma x\text{ (control)}$$

where Σx(control) = $\bar{x}$(control) x t (response duration, s).

Data are expressed in this way since measurement of $\Delta\Sigma x$ takes into account any variation in t as well as any change in $\bar{x}$ (see McQueen, 1977), and control experiments showed that control $\bar{x}$ does not vary appreciably over 10 - 20 min periods.

Dose-response data and presentation of data

Data from more than one experiment were usually pooled and expressed as the mean ± s.e. mean. In order to determine whether changes observed were statistically significant, particularly when they were small, responses to particular drug doses in the different experiments were compared with the corresponding responses to the drug vehicle in the same experiments using either the Wilcoxon signed ranks test (when the number of pairs $\geq$ 7) or Student's paired t test (for $\leq$ 6 pairs, and assuming Gaussian distribution) (see Colquhoun, 1971).

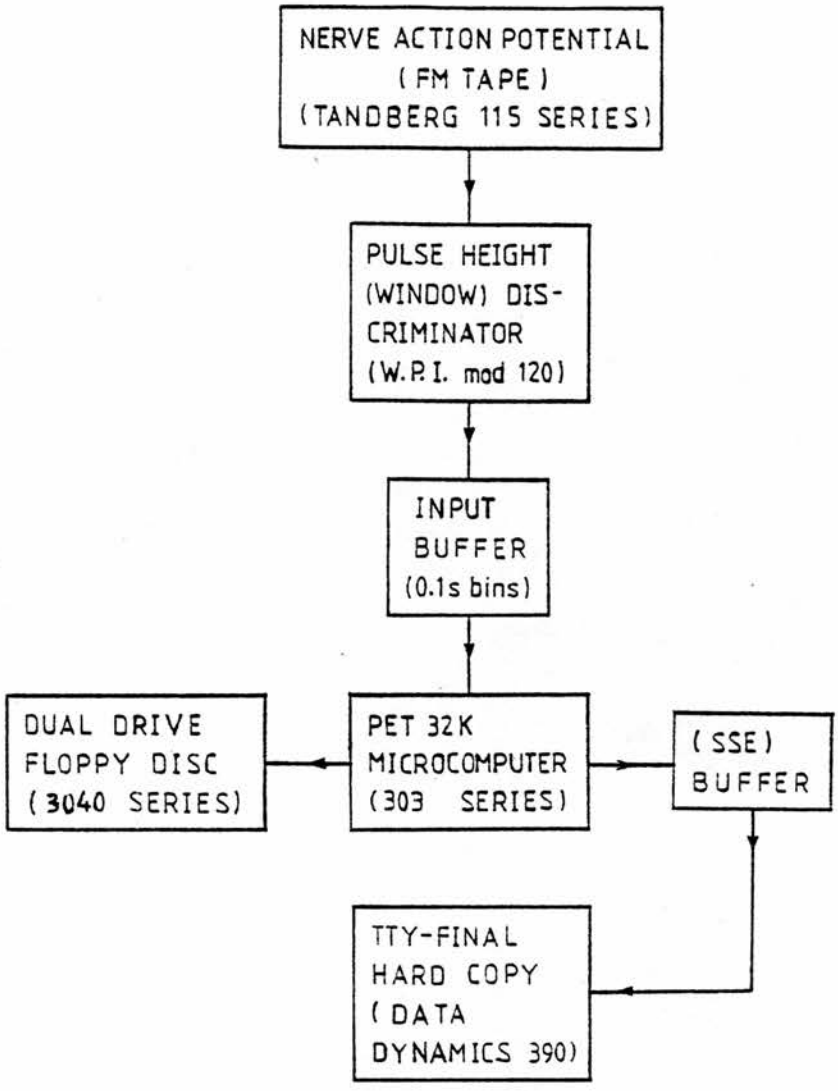


FIGURE 2.2

Schematic representation of the system used to analyse data. The information (chemoreceptor discharge) stored on a FM tape was fed to a PET 32K microcomputer and then stored on a floppy disk for subsequent analysis.

The null hypothesis was rejected if $P < 0.05$ and the difference between groups considered to be statistically significant.

Whenever possible, responses to a range of doses of test drug were obtained and $\Delta\Sigma x$ plotted against $\log_{10}$ dose. The portion of the dose-response curve over which the relationship between dose and response was approximately linear was determined and a straight line was fitted to the points using the method of least squares.

Linearly related dose-response data was obtained whenever possible. However, owing to the limited and somewhat unpredictable duration of a given recording it was considered expedient, in some cases, to administer test drugs in single doses before and after administration of another drug. The usefulness of data derived from single dose studies of this type was limited inasmuch as subtle changes in responsiveness were difficult to detect and dose-ratios could not be calculated. Nevertheless data of this type was considered a useful supplement to linearly-related dose-response data obtained for other drugs in the same experiment.

Drugs

Drugs were prepared in modified Locke solution (NaCl 6.0 g; KCl 0.42 g; $CaCl_2$ 0.24 g; Tris base 6.0 g; N-HCl 39 ml; distilled water to 1.0 l; pH 7.41 at 37°C, 295 m osmoles) or 0.9% w/v aqueous sodium chloride solution (297 m osmoles). Exceptions were dipyridamole which was initially dissolved in polyethyleneglycol 200, and β-endorphin in 0.5% aqueous bovine serum albumin solution, then both were diluted with saline. Doses referred to are those of the salts.

The drugs used in this work were: pentobarbitone sodium, gallamine triethiodide, morphine sulphate (May & Baker), acetylcholine

iodide, atropine sulphate, physostigmine salicylate, sodium cyanide (B.D.H.), dopamine hydrochloride (Koch Light), α(cis)-flupenthixol dihydrochloride (Lundbeck & Co.), mecamylamine hydrochloride (M.S.D.), naloxone hydrochloride (Endo), adenosine, adenosine 5'-monophosphate, adenosine 5'-diphosphate, adenosine 5'-triphosphate, coenzyme A, α-β-methylene adenosine 5'-triphosphate, β-γ-methylene adenosine 5'-triphosphate, cyclic AMP, dibutyryl-cyclic AMP, adenine, inosine, guanosine, cytidine, uridine, N^6-methyladenosine, 2'-chloro-adenosine, 3'-deoxyadenosine, 2'-deoxyadenosine, theophylline, aminophylline, imidazole, methionine-enkephalin, leucine-enkephalin, substance P, ouabain octahydrate (Sigma), cholecystokinin octapeptide non-sulphated (Peninsula Labs), human β-endorphin (Aalton Bio Reagents, Dublin), porcine β-endorphin (kindly supplied by Dr. D. Smyth, National Institute for Medical Research, London), vasoactive intestinal polypeptide (kindly supplied by Dr. S.I. Said, University of Texas Health Science Center, Dallas, U.S.A.), dihydro-ouabain (Boheringer Manheim), dipyridamole (Boheringer Ingelheim).

SECTION III

The Effects of Purines

INTRODUCTION

It has been shown that ATP is present in the carotid body (Nada and Ulano, 1972) and high concentrations of the nucleotide were found within specific granules of the type I cells stored together with catecholamines and proteins (Böck, 1980).

In view of the suggestions that ATP and adenosine, a metabolite of ATP, may play roles in neurotransmission (see e.g. Burnstock, 1972; Ribeiro, 1978), the opportunity was taken to investigate the action of adenosine and ATP on the carotid body chemoreceptors. Adenosine antagonists such as theophylline and aminophylline, adenosine uptake blockers such as dipyridamole, ATP, and adenosine analogues were also studied in order to characterize the purine receptors involved. Cyclic AMP and dibutyryl cyclic AMP were used to provide some information concerning the action of these compounds on arterial chemoreceptors.

RESULTS

Adenosine

Injections of adenosine

The effects of intracarotid injection of different doses of adenosine on spontaneous chemoreceptor discharge are illustrated in Figure 3.1. Discharge for each test was averaged over 10 s periods commencing 10 s before and continuing for 60 s after the injection. As can be seen from either the neurograms or the averaged discharge, the main effect was a dose-dependent increase in the frequency of discharge. No tachyphylaxis to the action of adenosine was observed. Thus, when injected at intervals of 5 min over a period of 1 h, there was no diminution of the effect on spontaneous chemoreceptor discharge.

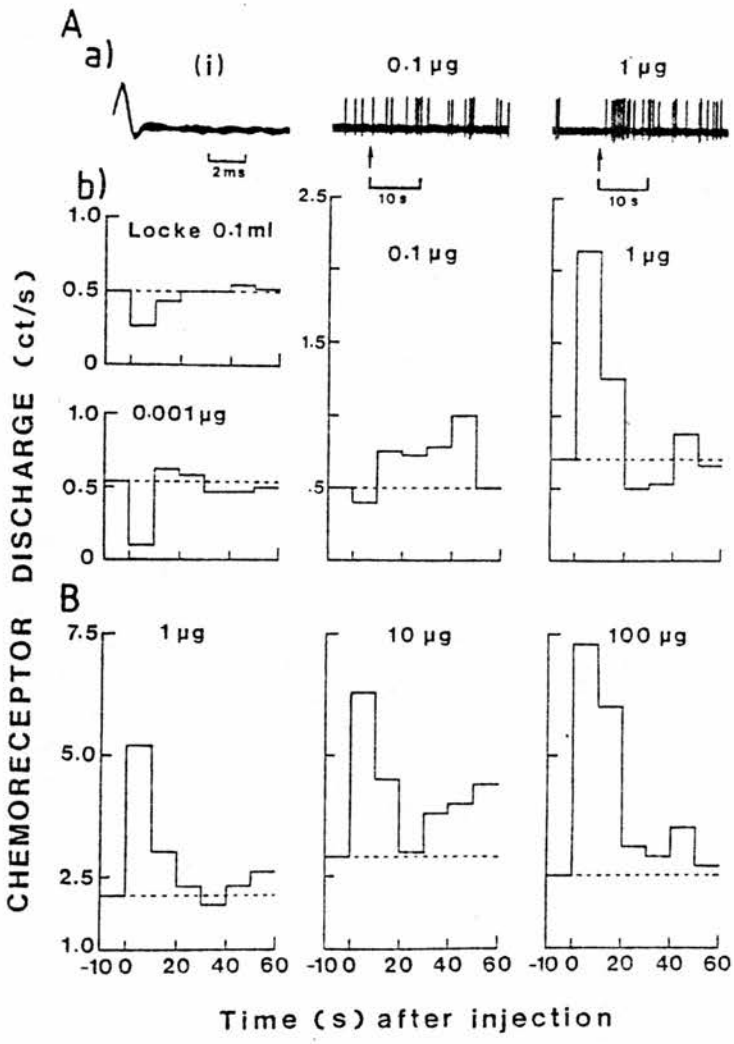


FIGURE 3.1

Effects of intracarotid (i.c.) injections of adenosine on the frequency of spontaneous chemoreceptor discharge. A. a) Neurograms taken from one experiment show the increase in the discharge of a single unit (insert, i) caused by injecting (arrow) adenosine 0.1 and 1 µg; in (i) the duration of the action potential is shown, the trace being of 10 consecutive superimposed spikes. b) Responses of the chemoreceptor unit to i.c. injection of 0.1 ml of the Locke solution flushed in by 0.2 ml of Locke solution (total Locke solution injected 0.3 ml), and to adenosine: 0.001 µg; 0.1 µg and 1 µg. B. Responses of three chemoreceptor units to adenosine: 1 µg; 10 µg and 100 µg.

The graphs show the intensity and duration of the responses averaged over 10 s periods following the injections.

Low doses of adenosine (0.001 - 0.01 μg) caused a decrease in chemoreceptor discharge during the first 10 s following the injection (see Figure 3.1), an effect which appeared to be most pronounced for the lowest dose. However, a slight inhibition of spontaneous discharge was also usually associated with injection of the same volume of drug vehicle, Locke solution (see Figures 3.1 and 3.2), so responses to the low doses of adenosine were studied (see Figure 3.2) and compared with responses to injections of Locke solution (0.3 ml). There was no significant difference ($P > 0.05$) between the responses to adenosine and Locke solution in these experiments.

Data obtained from ten experiments were averaged over 5 s periods for 30 s after the injection, pooled and plotted against time (Figure 3.2). This quantitative evidence confirmed that the increases in discharge were dose-related. The peak of the discharge occurred between 5 and 10 s after the injections for all doses (0.1 - 100 μg), although that caused by 0.1 μg was not significantly different from that caused by the highest dose studied, 100 μg ($P > 0.05$). The increase caused by adenosine (1 - 100 μg) was significantly different from the control ($P < 0.05$) in the first 5 s following the injections. The threshold dose for the adenosine excitatory action is thus between 10 and 100 ng. Injections of Locke solution were also made in these experiments and the results obtained summarized in Figure 3.2. They confirm the transient inhibitory effect of Locke solution illustrated in Figure 3.1. A significant decrease in discharge occurred during the first 5 s following the injection ($P < 0.05$, compared with the pre-injection averaged discharge). This was much less marked and not statistically significantly different in the next 5 s ($P > 0.05$), and thereafter discharge returned to the pre-injection control levels.

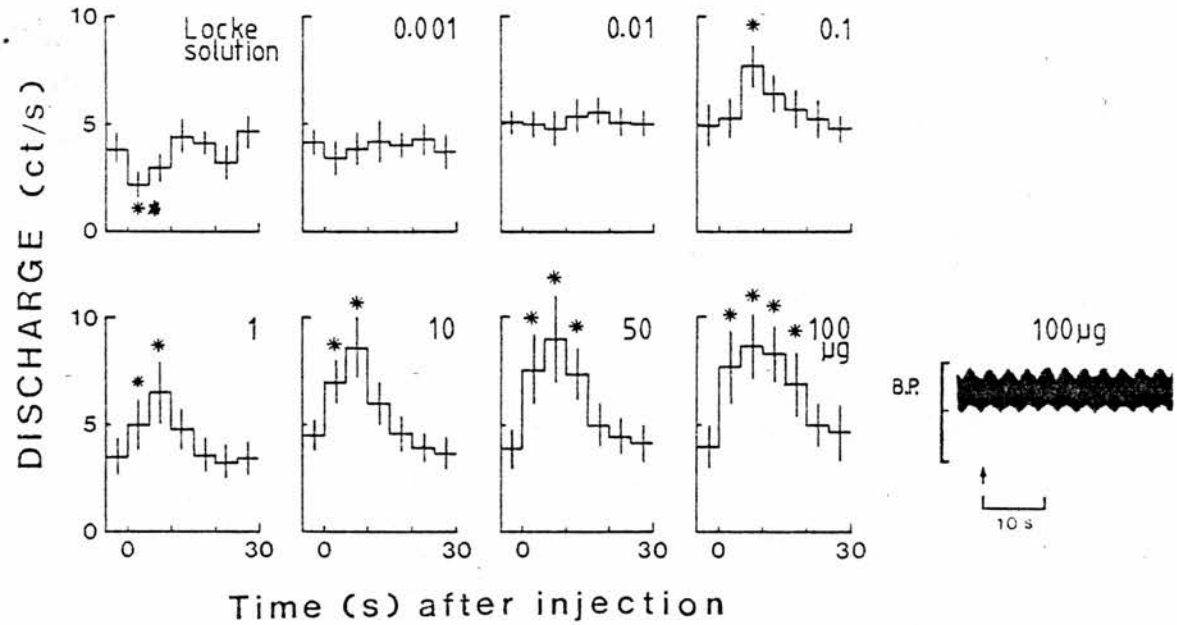


FIGURE 3.2

Effects on spontaneous chemoreceptor discharge (ct/s) of injecting Locke solution (0.3 ml, see legend of Figure 3.1) and adenosine (0.001; 0.01; 0.1; 1; 10; 50 and 100 µg). Discharge was averaged over 5 s periods following the injection. Data obtained from ten cats were pooled and presented as the mean with vertical bars indicating s.e. mean. The panel on the right shows an arterial blood pressure (B.P.) trace after injecting (arrow) 100 µg of adenosine in one experiment. B.P. calibration, 0-100-200 mmHg.

* P <0.05 compared with Locke solution injections.
** P <0.05 compared with pre-injection (control) period.

Results from four experiments in which both average discharge and maximum discharge, expressed as ct/s, were obtained in response to different doses of adenosine are compared and summarized in Table 3.1. The average spontaneous chemoreceptor discharge was determined in the 15 s pre-injection control period immediately preceding each injection and in the 15 s post-control period commencing 45 s after the injections. Responses to adenosine were evaluated by determining the average discharge in the period during which discharge was increased above control frequency. Apart from increasing the average discharge, adenosine also increased the maximum discharge frequency. This latter increase, however, was not so marked as that averaged over the whole period, showing that the overall increase in discharge caused by adenosine resulted from a sustained increase throughout the response rather than from a sudden transient increase, such as occurs with ACh (cf. McQueen, 1977). The duration of the adenosine response was dose-dependent, and the delay to onset of the response was inversely related to dose.

The effects obtained were not related to changes in systemic blood pressure since adenosine in the doses studied did not cause any consistent or substantial changes in arterial blood pressure, as can be seen from Figure 3.2. Neither were changes in arterial blood gas tensions nor pH observed following administration of adenosine.

Infusions of adenosine

Adenosine was infused at a rate of 50 μg/min for 2 min; six infusions were performed in two cats. A sustained increase in chemoreceptor discharge occurred, which returned to pre-infusion control levels within 30 s of stopping the infusion (Figure 3.3). The peak response was significantly different from the control, whether this was

TABLE 3.1: Effect of adenosine on chemoreceptor discharge in the cat.

ADENOSINE	*CHEMORECEPTOR DISCHARGE*						*RESPONSE*	
	Average discharge			*Maximum discharge*			*Duration*	*Delay to onset*
(μg) (No. of expts)	Pre-control* (=100%) (ct/s) Mean, range	Effect of adenosine (% of control) Mean, range	Post-control** (=100%) (ct/s) Mean, range	Pre-control† (=100%) (ct/s) Mean, range	Effect of adenosine (% of control) Mean, range	Post-control†† (=100%) (ct/s) Mean, range	(s) Mean, range	(s) Mean, range
0.1 (4)	6.1 *5.2-6.9*	156 *108-184*	5.1 *3.9-5.8*	12 *11-14*	135 *73-181*	11 *8-13*	8.0 *6.0-11.7*	7.1 *3.2-10.1*
1 (3)	4.9 *3.6-6.2*	181 *172-191*	5.0 *3.7-7.1*	10 *8-12*	142 *70-206*	11 *8-12*	12.7 *9.0-15.4*	5.4 *2.1-11.3*
10 (4)	4.0 *1.9-5.1*	209 *195-218*	3.6 *1.3-4.7*	9 *5-12*	175 *143-196*	10 *6-13*	13.8 *12.1-14.6*	4.7 *2.1- 8.2*
50 (3)	3.6 *1.9-5.1*	248 *247-249*	3.8 *2.1-4.7*	7 *5-10*	209 *183-223*	9 *6-11*	15.1 *13.7-16.5*	3.6 *0.7- 8.4*
100 (3)	5.5 *1.8-7.6*	229 *221-243*	5.5 *2.8-6.9*	13 *5-17*	209 *180-240*	12 *7-15*	19.8 *14.3-25.4*	1.7 *0.5- 3.0*

* Discharge averaged during the 15 s immediately before injecting adenosine

** Discharge averaged during the 15 s period commencing 45 s after the injection, by which time the discharge had usually returned to pre-injection levels. There was no significant difference between pre- and post-control values ($P > 0.05$).

† Maximum discharge in 1 s observed during the pre-control period.

†† Maximum discharge in 1 s observed during the post-control period.

taken as the pre-infusion discharge or the discharge averaged during an infusion of the same volume of the vehicle Locke solution (0.1 ml/min). The infusion of Locke solution had no appreciable effect on spontaneous discharge (Figure 3.3). Adenosine infusions were not associated with changes in systemic blood pressure, as can be seen from Figure 3.3, or with marked changes in arterial blood gas tensions or pH (see legend of Figure 3.3).

Adenosine triphosphate

ATP (1 - 100 μg i.c.) increased the spontaneous chemoreceptor discharge (see Figures 3.4 and 3.5). Data obtained from six experiments were pooled and expressed in ct/s ± s.e. mean and plotted as a function of time during the 50 s period following the injections (Figure 3.5). Control injections of Locke solution (0.3 ml) were carried out in all experiments; they caused a slight decrease in spontaneous chemoreceptor discharge in the first 10 s after injection followed by slight oscillations (increases and decreases) in the discharge. Statistical analysis of the results was carried out by comparing the frequency of the discharge averaged in 5 s periods after injecting ATP with that averaged during the same periods following injection of Locke solution. The comparison using Student's paired t-test showed that ATP significantly increased the discharge between 5 and 10 s, after injecting ATP 10 μg, and between 5 and 15 s after ATP 100 μg ($P < 0.05$) (Figure 3.5). The effect was dose-dependent and the response lasted 25 to 30 s for the higher dose. Recovery to the control levels occurred for the higher dose in the next 20 s, i.e. in 50 s after the injection.

No tachyphylaxis to the action of ATP was observed, thus when injected at intervals of 5 min over a period of 1 hr, there was no diminution of the effect on spontaneous chemoreceptor discharge.

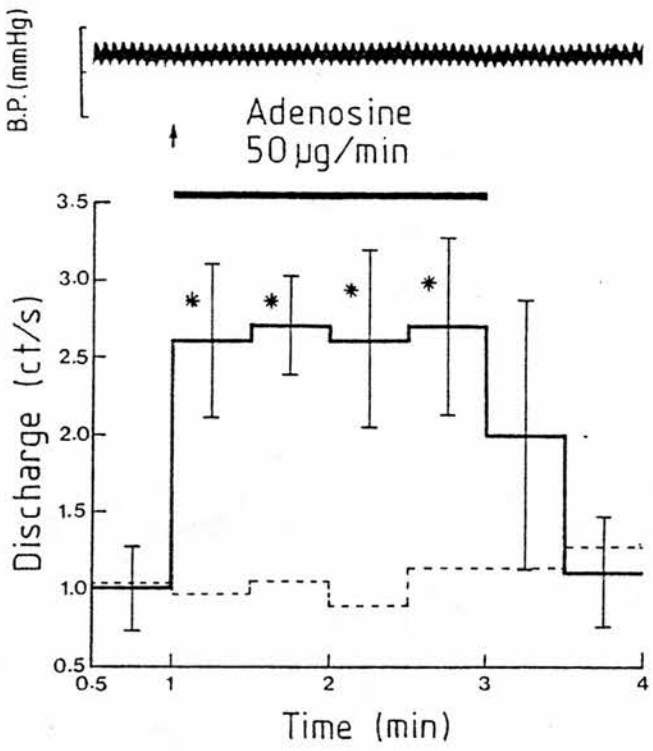


FIGURE 3.3

Pooled data from 6 infusions of adenosine (50 µg/min i.c.) in two cats showing the effect on spontaneous chemoreceptor discharge. The broken line represents the typical discharge during an infusion of Locke solution (0.1 ml/min). The horizontal bar indicates the duration of infusions. The upper panel shows a blood pressure trace recorded during one of the adenosine infusions. B.P. calibration is 0-100-200 mmHg. Arterial blood samples taken during an adenosine infusion gave values of pH 7.36, PaO_2 105 Torr, $PaCO_2$ 30 Torr, compared with pre-infusion sample values of pH 7.35, PaO_2 105 Torr, $PaCO_2$ 30 Torr.

* $P < 0.05$ paired t-test, compared with pre-infusion control period.

The effect was not associated with changes in the arterial blood pressure as can be seen from a blood pressure trace illustrated in Figure 3.10.

Comparison between the effects of ATP, ADP, AMP and adenosine

A close comparison between the effects of ATP and adenosine was carried out in six cats. The results from these experiments are summarized in Figure 3.6, chemoreceptor responses were expressed as the increase in the discharge in the 30 s immediately following injections. Discharge, expressed as $\Delta\Sigma x$, was plotted against dose expressed in moles. The results show that both substances caused similar quantitative effects when compared on a molar basis adenosine (m.w. ≈ 267), ATP (m.w. ≈ 507). However, the effect of adenosine had a faster onset, for example, adenosine (1 - 100 µg) caused increases in chemoreceptor discharge statistically significantly different from the Locke solutions in the first 5 s after injection (Figure 3.2), whereas although ATP caused a significant increase, it occurred only in the period between 5 and 10 s following the injection for the doses of 10 - 100 µg (Figure 3.5).

The effects of ADP and AMP were also investigated in two cats and compared with those of adenosine and ATP (Figure 3.7). All four substances (1 - 100 µg i.c.) caused a dose-related increase in spontaneous chemoreceptor discharge but the effects of lower doses of ATP, ADP and AMP were smaller than those obtained with adenosine. However, if those doses are expressed in moles the substances appear to have an activity which is very similar to that of adenosine.

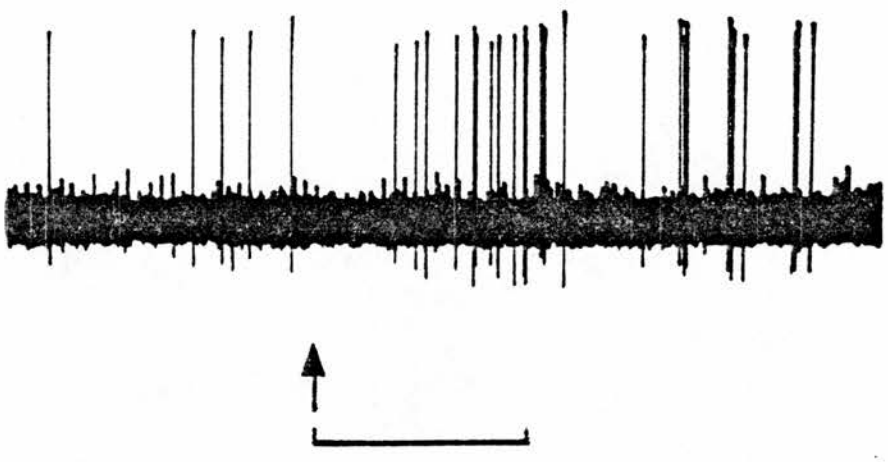


FIGURE 3.4

Recording of a single chemoreceptor unit illustrating the response of ATP (1 µg i.c.). Panel shows from above downwards: action potentials, injection marker and 10 s calibration.

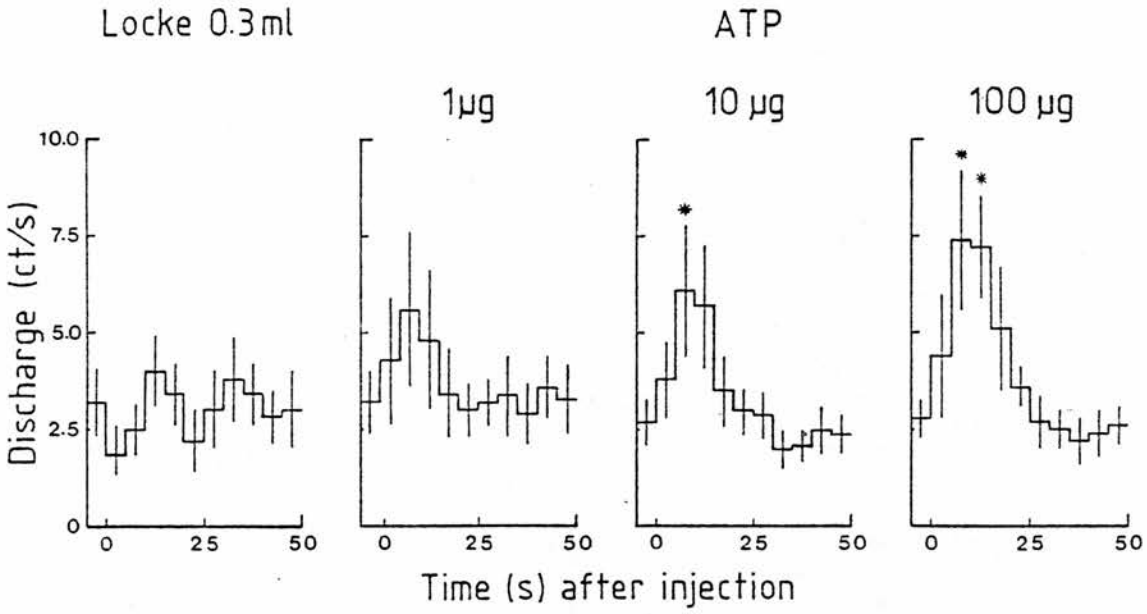


FIGURE 3.5

Effects on spontaneous chemoreceptor discharge (ct/s) of injecting Locke solution (0.3 ml, 0.1 ml + 0.2 ml to flush in, see legend of Figure 3.1) and ATP 1 µg, 10 µg and 100 µg i.c. Discharge was averaged over 5 s periods following the injections. Data obtained from tests in six cats were pooled and presented as the mean ± s.e. mean.

* P <0.05 (paired t-test) compared with Locke solution injections.

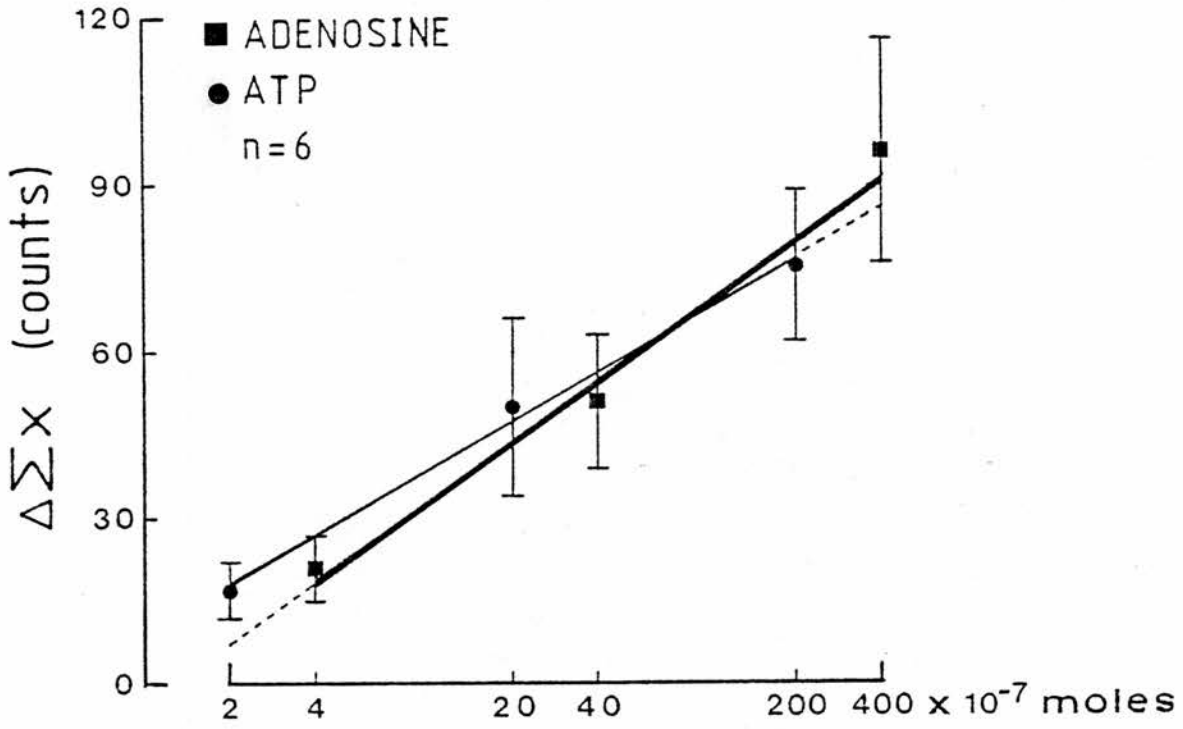


FIGURE 3.6

Comparison of ATP with adenosine on spontaneous chemoreceptor activity. Each point is the average ± s.e. mean of six experiments expressed as the increase on spontaneous discharge occurring in the 30 s and following the injections. The broken lines are extrapolations. Lines were fitted to the data by the method of least squares. Averaged values ± s.e. mean (ct/s) for the pre-injection (15 s) control periods are as follows: adenosine 2 x 10^{-7} moles 3.7 ± 0.9; 20 x 10^{-7} moles 4.2 ± 0.9 and 200 x 10^{-7} 3.9 ± 1.2; ATP 4 x 10^{-7} moles 3.2 ± 0.8, 40 x 10^{-7} 2.7 ± 0.6 and 400 x 10^{-7} moles 3.2 ± 0.9. Chemoreceptor responses are expressed as the mean $\Delta\Sigma x$ (see Section II, Methods and Materials) calculated from the response during the 30 s period immediately following the injections.

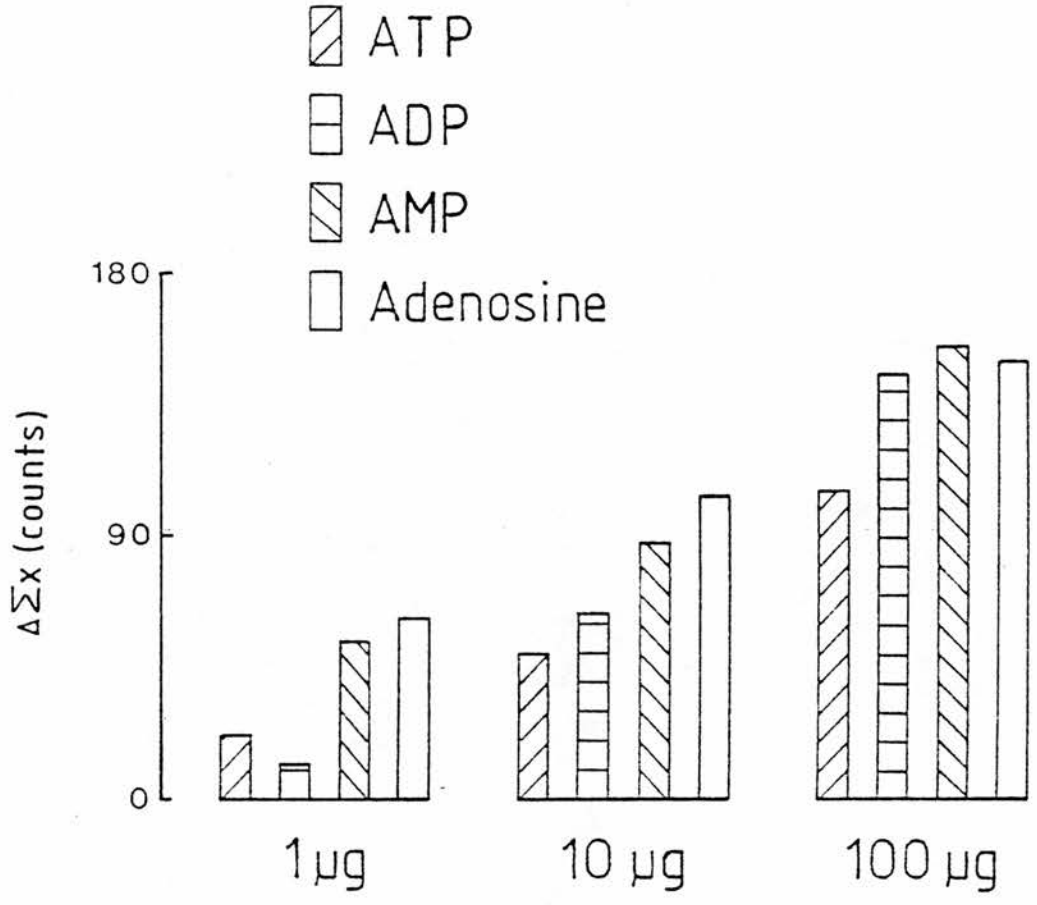


FIGURE 3.7

Comparison between the effects of ATP, ADP, AMP and adenosine on spontaneous chemoreceptor discharge in two cats (recordings from a total of 2 - 3 chemoreceptor units). Averaged values (ct/s) for the pre-injection (15 s) control periods are as follows: ATP 1 µg 1.1, 10 µg 3.2, 100 µg 1.7; ADP 1 µg 5.1, 10 µg 3.3, 100 µg 3.6; AMP 1 µg 3.2, 10 µg 6.3, 100 µg 4.6; adenosine 1 µg 1.6, 10 µg 3.0, 100 µg 6.7.

Coenzyme A

In view of the suggestion that coenzyme A (CoA), a naturally occurring adenine compound, could play a role in neurotransmission (Cook, Hamilton and Okwuasaba, 1978), an investigation was undertaken to determine whether CoA affected carotid body chemoreceptor activity and to compare its effects with those of ATP and adenosine in the same animal.

Results from one of the experiments in which CoA was studied (1 - 100 µg i.c.) are illustrated in Figure 3.8. CoA increased chemoreceptor discharge in a dose-dependent manner and its effect was similar to that caused by both adenosine and ATP.

Since the effect of CoA was not markedly different from that of ATP or adenosine its activity is probably related to the adenine nucleotide portion of the molecule.

ATP in the presence of adenosine and adenosine in the presence of ATP

In order to test whether ATP acted in the same way as adenosine an experiment was performed in which ATP (100 µg) was injected i.c. during an infusion of adenosine (5 µg/min i.c.). As can be seen in Figure 3.9, the presence of adenosine reduced the full chemoreceptor response to ATP, such was obtained before starting the infusion of adenosine.

In another experiment adenosine (50 µg i.c.) was injected 20 min after starting an ATP infusion (10 µg/min i.c.). The excitatory response to adenosine was reduced from $\Delta\Sigma x$ = 55 counts before starting the ATP infusion to $\Delta\Sigma x$ = 18 counts during the infusion.

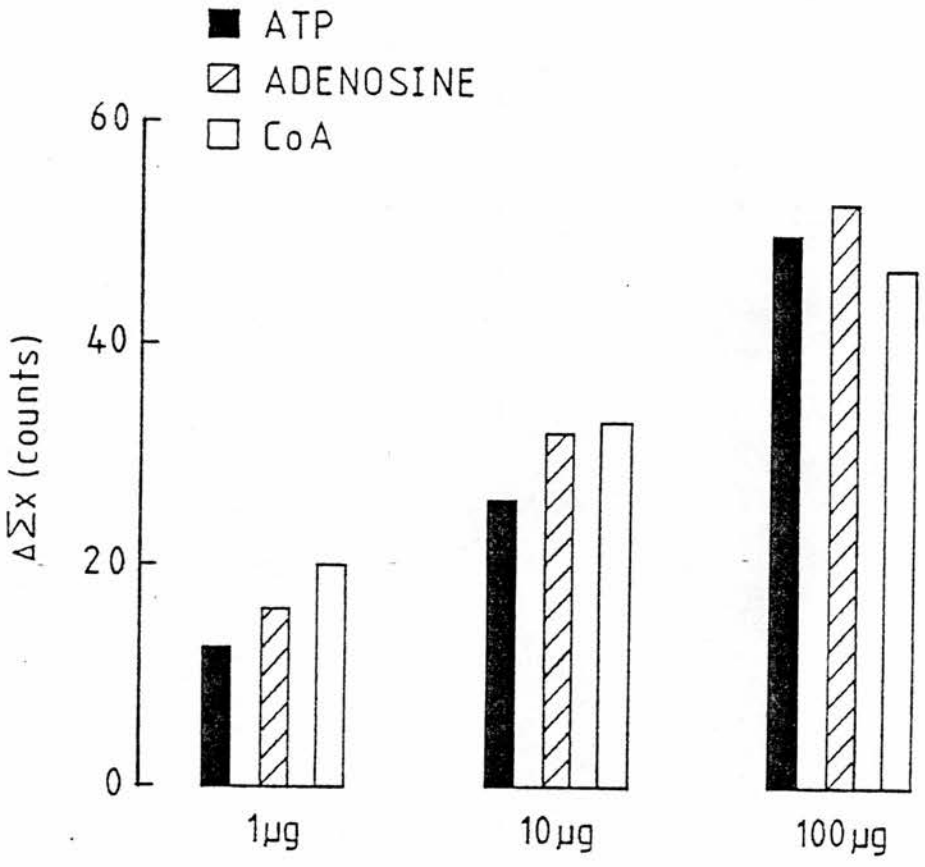


FIGURE 3.8

Effects of coenzyme A (CoA) on chemoreceptor activity and its comparison with ATP and adenosine in a cat (recordings from 3 - 4 chemoreceptor units). Values (ct/s) for the pre-injection (15 s) control period are as follows: ATP 1 µg 1.5, 10 µg 1.8, 100 µg 2.0; adenosine 1 µg 1.7, 10 µg 1.3, 100 µg 2.4; CoA 1 µg 1.6, 10 µg 1.7, 100 µg 3.5.

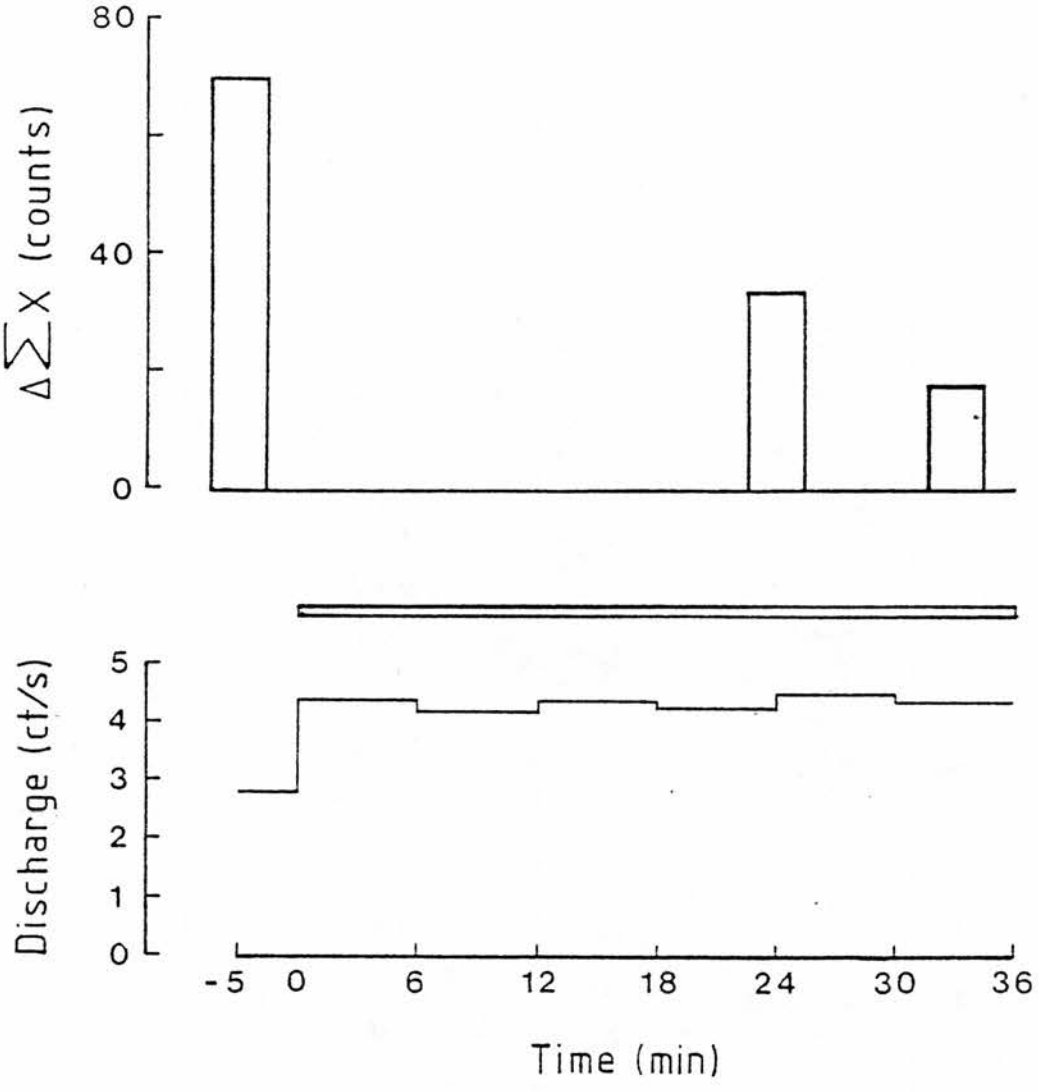


FIGURE 3.9

Effect of ATP on chemosensory discharge (recordings from 2 - 3 chemoreceptor units) in the presence of an adenosine infusion. The upper panel shows from left to right the responses to ATP (100 µg i.c.) obtained immediately before starting the infusion and at 25 and 35 min. The lower panel shows the increase in spontaneous chemoreceptor discharge observed after starting adenosine infusion (5 µg/min i.c.). The horizontal bar indicates the duration of the infusion.

ATP analogues: α- β-methylene ATP and β-γ-methylene ATP

The bigger delay to onset of response seen with ATP, in comparison with adenosine (see Figures 3.2 and 3.5) suggests that the effect of ATP might result from it being hydrolysed to adenosine. To test this further the effects of the non-hydrolysable ATP analogues: α-β-methylene ATP and β-γ-methylene ATP were investigated. The effects of these substances were compared with ATP in four cats and the results are summarized in Figure 3.10. In contrast to the effects of ATP, α-β-methylene ATP (100 μg i.c.) caused a decrease in spontaneous chemoreceptor discharge; the effect was associated with an increase in arterial blood pressure. The effect of β-γ-methylene ATP (100 μg i.c.) was not so clear-cut since in one experiment it caused an effect similar to that of ATP itself while in another three experiments it caused a smaller increase in discharge. Averaging the four experiments the mean increase was not statistically significantly different either from the Locke solution injections or from the pre-injection discharge ($P > 0.05$).

Dipyridamole

The effect on spontaneous chemoreceptor discharge of infusing dipyridamole (50 μg/min i.c.) is illustrated in Figure 3.11. The substance increased the discharge during the period of infusion (20 min), discharge returning to control values 10 min after stopping the infusion.

The effects of lower doses (1 μg i.c.) of both adenosine and ATP were potentiated during dipyridamole infusion (50 μg/min i.c.) but the effects induced by higher doses (100 μg i.c.) were reduced and the chemoexcitation caused by adenosine was converted into a biphasic response with an early inhibitory component (see Figure 3.12).

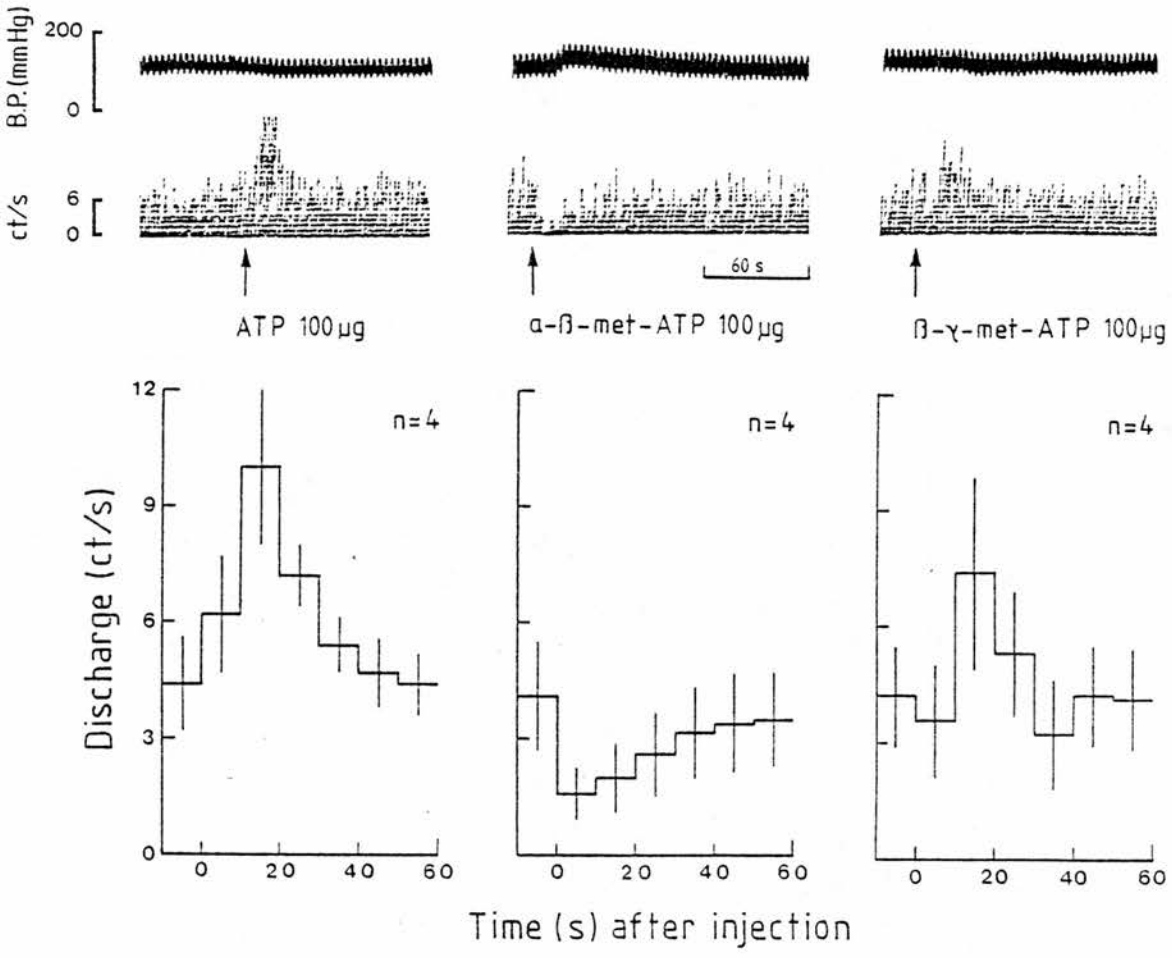


FIGURE 3.10

Comparison of the effects of non-hydrolysable ATP analogues, α-β-methylene ATP and β-γ-methylene ATP with ATP on spontaneous chemoreceptor discharge (ct/s). The upper panel shows the effects on arterial blood pressure (B.P.) and of injecting ATP, α-β-methylene ATP and β-γ-methylene ATP on spontaneous discharge in the same cat (recordings from 3 - 4 chemoreceptor units). The lower panel shows averaged data obtained from four experiments.

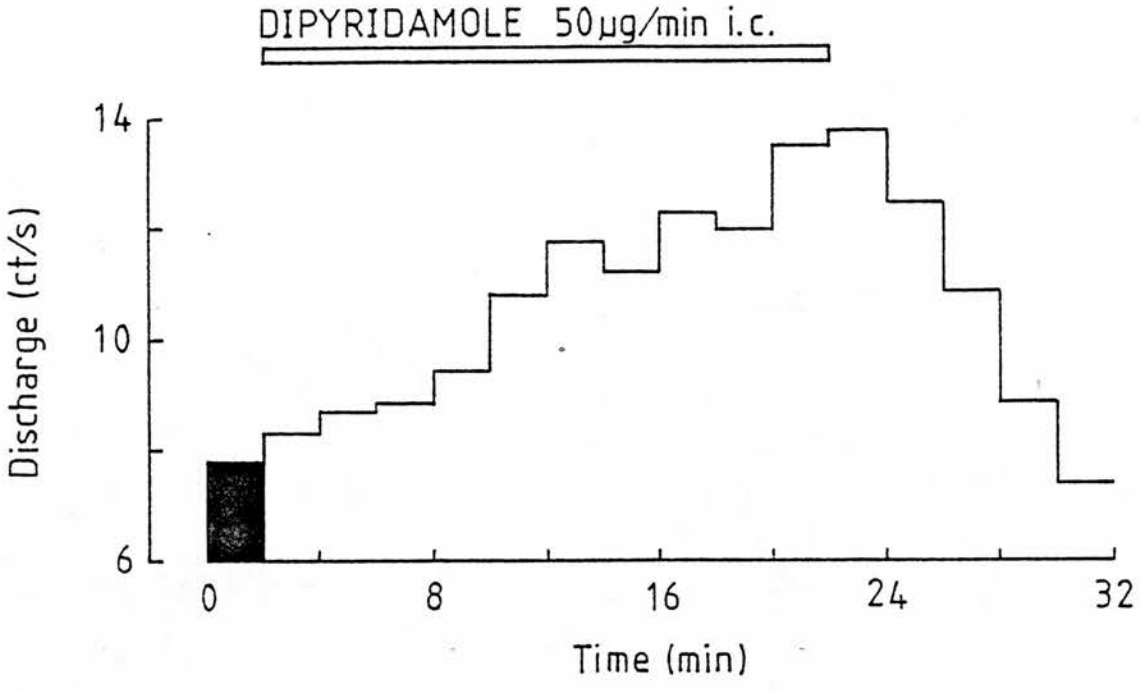


FIGURE 3.11

Effect of dipyridamole on spontaneous chemoreceptor discharge (ct/s). Horizontal bar indicates duration of infusion. The black rectangle indicates the average discharge in the 2 min that preceded the infusion. Recordings from 3 - 4 chemoreceptor units.

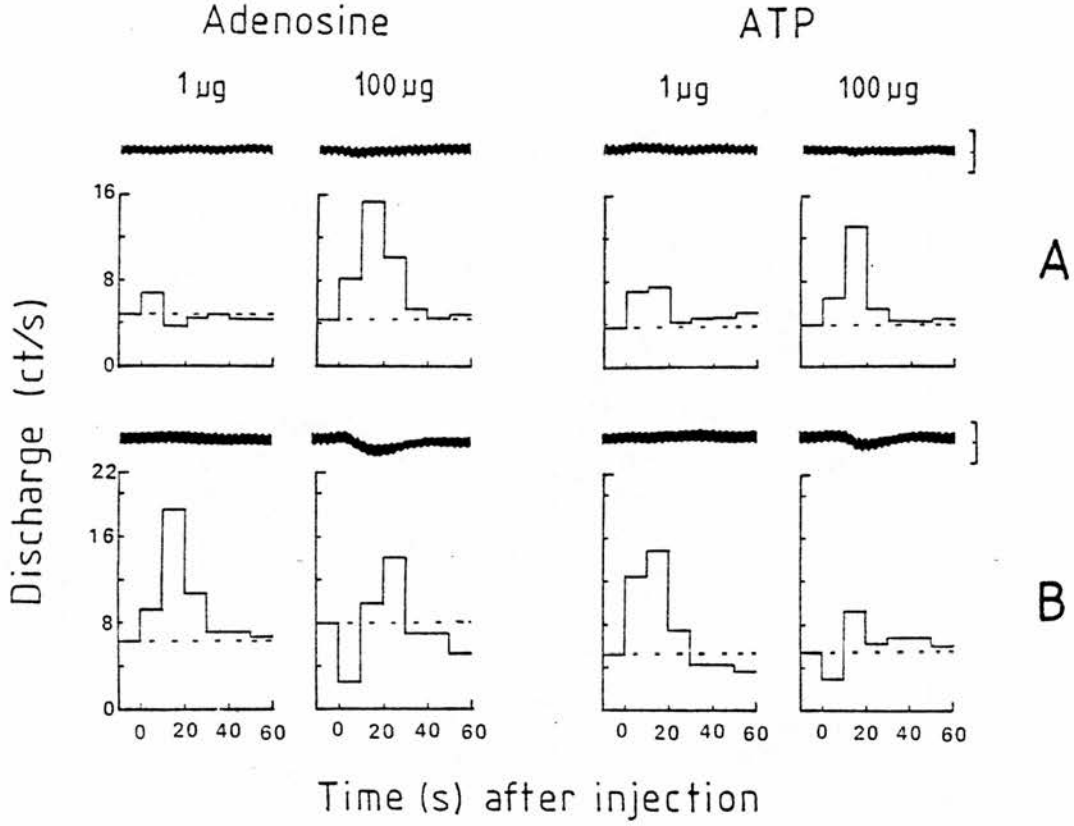


FIGURE 3.12

Effects of adenosine (i.c.) and ATP (i.c.) on spontaneous chemoreceptor discharge (ct/s) (recordings from 3 - 4 chemoreceptor units). A) before, and B) in the presence of dipyridamole (50 µg/min i.c.). Above each graph is shown the B.P. trace recorded after injecting the substances. B.P. calibration 0-100-200 mmHg.

Dose-response curves to adenosine (1 - 400 µg i.c.) were obtained before and after dipyridamole (50 µg/min i.v.) (Figure 3.13). As can be seen dipyridamole itself administered intravenously caused a slight increase in spontaneous chemoreceptor discharge during the period of infusion (10 min) and dose-response curves to adenosine were shifted upwards and to the left during dipyridamole intravenous infusion (Figure 3.13).

Specificity for adenosine

A number of neucleosides have actions on neurotransmission (e.g. Phillis, Edstrom, Kostopoulos and Kirkpatrick, 1979). So it was considered of interest to evaluate the effect of some of those substances on the carotid body chemoreceptor activity in order to explore the specificity of the adenosine receptor. Figure 3.14 summarizes results from two experiments in which adenine, the purine nucleosides inosine and guanosine, and the pyrimidine nucleosides cytidine and uridine were tested. The effects were compared with those of adenosine itself. As can be seen neither adenine (10 - 100 µg) nor the nucleosides, inosine, guanosine, cytidine or uridine, caused any substantial changes in the spontaneous chemoreceptor discharge, whereas adenosine caused the usual increase in discharge.

Evoked responses

Figure 3.15 shows responses to single doses of NaCN (5 µg i.c.), CO_2-equilibrated Locke solution (0.3 ml i.c.) ACh (50 µg i.c.) and dopamine (5 µg i.c.) obtained before and after (5 - 20 min) injections of adenosine (100 µg i.c.). The chemoexcitatory responses evoked by both NaCN and CO_2 were decreased after adenosine, the chemo-

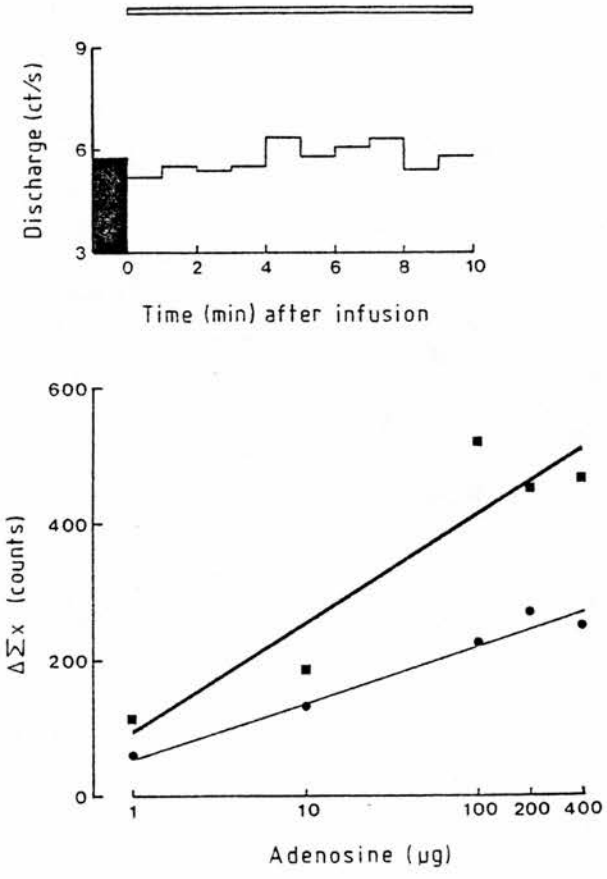


FIGURE 3.13

The upper part of the figure shows the effect of dipyridamole (50 μg/min i.v.) on spontaneous chemoreceptor discharge (ct/s) (recordings from 3 chemoreceptor units). The horizontal bar indicates the duration of the infusion and the black rectangle the discharge averaged in the 2 min that preceded the infusion. In the lower part the dose-response curves to adenosine (i.c.) obtained before (filled circles) and during (filled squares) a dipyridamole infusion are shown. Values (ct/s) for the pre-injection (15 s) control periods are as follows: adenosine 1 μg 2.7 before dipyridamole, 2.6 during dipyridamole infusion; 10 μg 2.0 before, 2.5 during; 100 μg 3.8 before, 3.3 during; 200 μg 3.4 before, 4.5 during; 400 μg 5.8 before, 4.8 during.

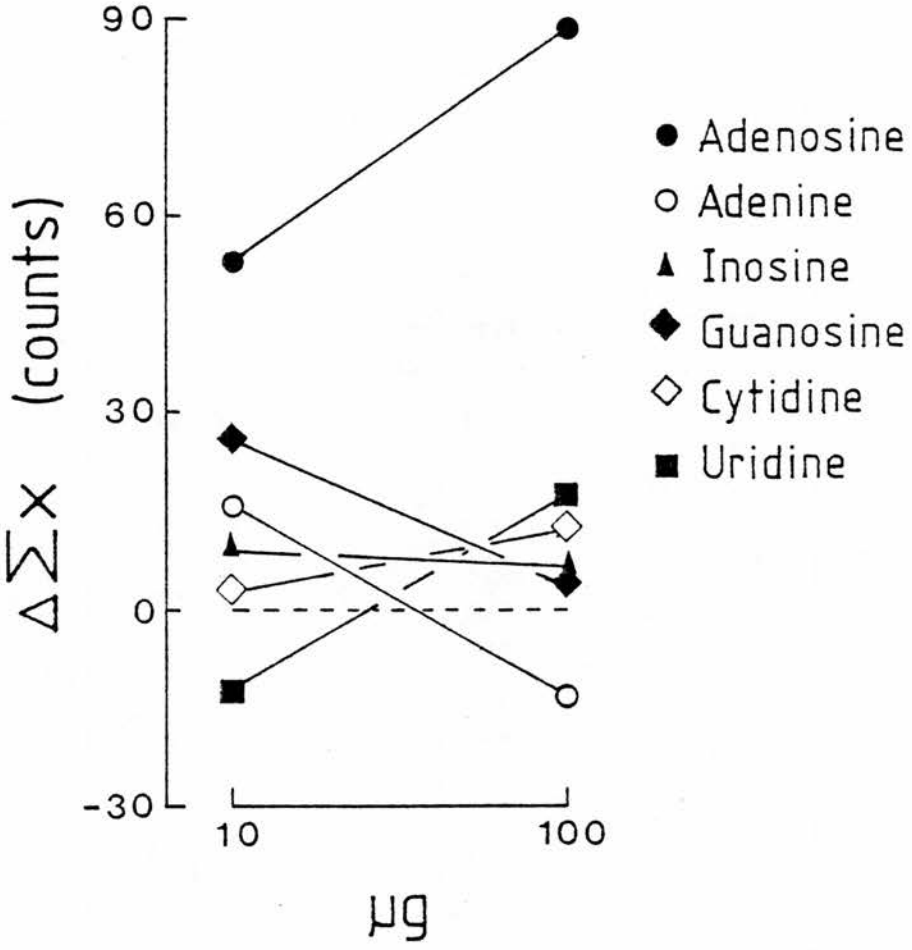


FIGURE 3.14

Comparison between the effects of adenosine, adenine, inosine, guanosine, cytidine and uridine in two cats (recordings from a total of 5 - 7 chemoreceptor units). Averaged values (ct/s) for the pre-injection (15 s) control periods for 10 and 100 µg respectively are as follows: adenosine 3.4, 3.0; adenine 2.3, 2.1; inosine 3.5, 3.2; guanosine 6.3, 7.8; cytidine 3.1, 2.7; uridine 1.5, 1.2.

excitatory response evoked by ACh was enhanced, and the chemo-inhibitory response evoked by dopamine was also increased. These single dose results are not so reliable as dose-response curve studies (but see Section II, Methods and Materials). In order to investigate further whether adenosine influenced the responses evoked by those substances, dose-response curves to NaCN (1 - 5 µg i.c.), ACh (10-50 µg i.c.) and dopamine (1 - 5 µg i.c.) obtained before and during adenosine infusions (10 µg/min i.c.) were performed in one experiment and the results are shown in Figure 3.16. The excitatory responses evoked by NaCN were not consistently changed during infusion of adenosine as compared with the pre-infusion responses, whereas both the chemoexcitatory responses evoked by ACh and the chemoinhibitory responses evoked by dopamine were enhanced during adenosine infusions.

Theophylline and aminophylline

Methylxanthines, particularly theophylline and aminophylline, have been considered as adenosine antagonists (for a review see e.g. Fredholm, 1980). It was decided to study the effects of theophylline and aminophylline on chemoreceptors and to determine whether these substances influenced the chemoreceptor responses to adenosine.

Theophylline (1 mg) was injected i.c. in five cats and caused a decrease in spontaneous chemoreceptor discharge, an effect which lasted about 30 s. The integrated response ($\Delta \Sigma x$) showed that the depression was significantly greater ($P < 0.05$) than that caused by injecting Locke solution (Figure 3.17). Although theophylline transiently decreased spontaneous chemoreceptor discharge, it did not prevent the excitatory action of adenosine, and as can be seen in Figure 3.18, the log dose-response curve to adenosine was shifted upwards and to the

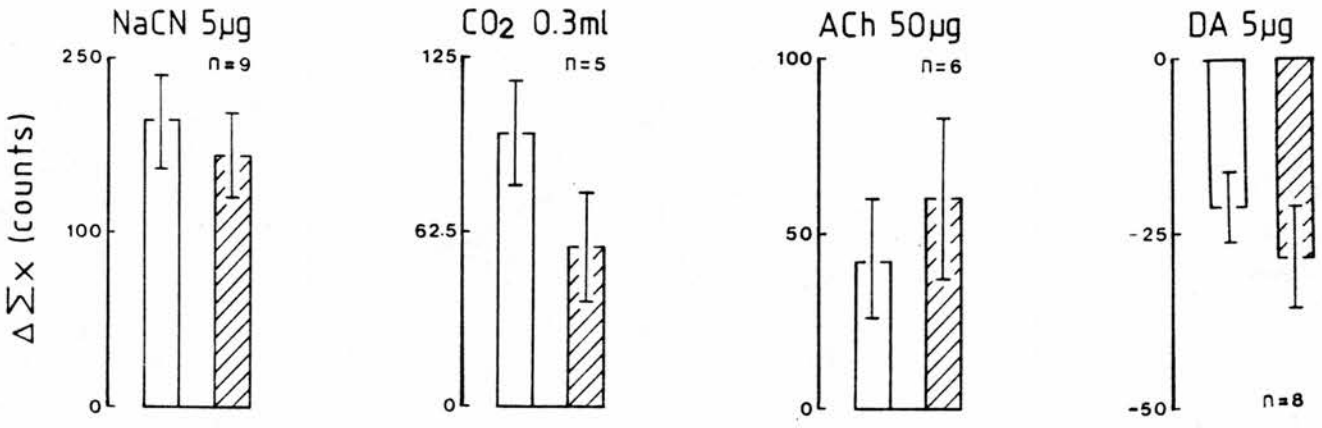


FIGURE 3.15

Effects of adenosine (100 µg i.c.) on the chemoreceptor responses evoked by NaCN, CO_2-equilibrated Locke solution (CO_2), acetylcholine (ACh) and dopamine (DA) before (□) and after (▨) adenosine injections. Averaged values (ct/s) for the pre-injection (10 - 15 s) control periods are as follows: NaCN before 5.5 ± 1.6, after 3.8 ± 0.6; CO_2 before 5.1 ± 1.4, after 5.3 ± 1.5; ACh before 3.9 ± 1.8, after 3.2 ± 1.2; DA before 2.2 ± 0.6, after 3.0 ± 1.2.

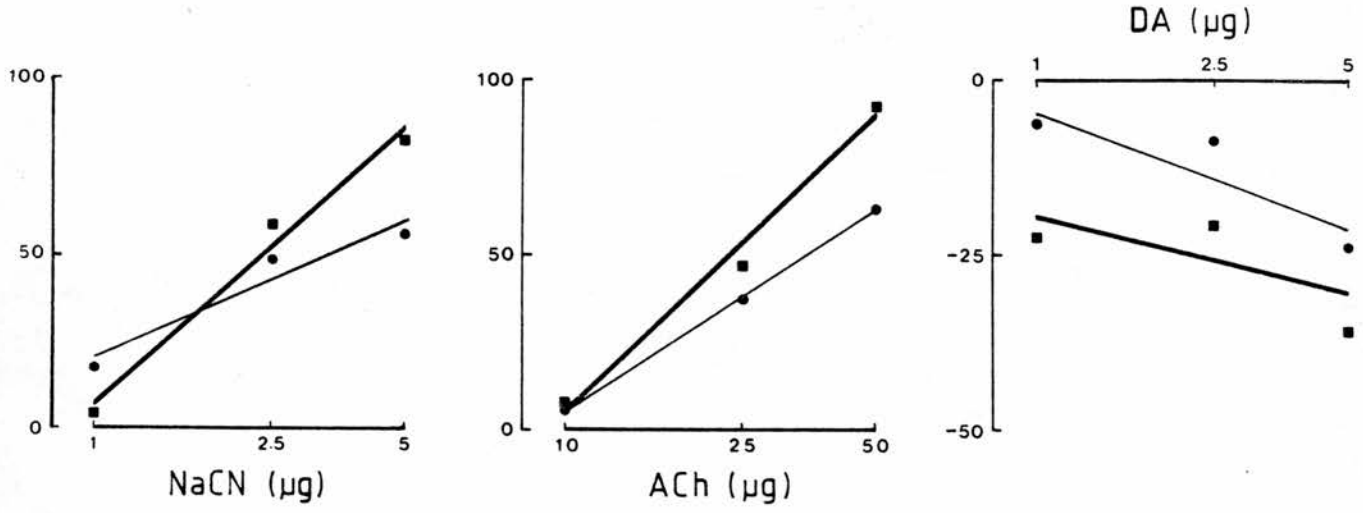


FIGURE 3.16

Dose-response curves to NaCN, ACh and dopamine (DA) i.c. obtained before (filled circles) and during (filled squares) an adenosine infusion (10 µg/min i.c.) in one experiment (recordings from a single chemoreceptor unit). Lines were fitted to the data by the method of least squares. Averaged values (ct/s) for the pre-injection (10-15 s) control periods are as follows: NaCN before adenosine infusion 1 µg 3.5, 2.5 µg 2.1, 5 µg 3.1; during infusion 1 µg 4.0, 2.5 µg 3.7, 5 µg 2.1; ACh before 10 µg 4.1, 25 µg 3.1, 50 µg 2.3; during infusion 10 µg 2.2, 25 µg 5.1, 50 µg 6.8; DA before 1 µg 0.7, 2.5 µg 0.7, 5 µg 2.1; during infusion 1 µg 3.6, 2.5 µg 3.4, 5 µg 2.9.

left which is indicative of an increase in the responses after theophylline; the response to adenosine 100 μg was statistically significantly greater after theophylline than it was before ($P < 0.05$, $n=4$).

Injections of lower doses of theophylline (100 - 200 μg i.c.) did not cause any significant effect on the spontaneous discharge ($P > 0.05$, $n=3$ pairs), and the excitatory effect of adenosine (1 - 100 μg) was unaltered by these doses ($P > 0.05$, $n=3$ pairs).

Aminophylline (1 mg i.c.) was injected in one cat and the effect of adenosine (1 - 100 μg) tested before and after the injection. A transient depressant effect similar to that seen with theophylline was observed, and the excitatory action of adenosine was also undiminished after aminophylline.

Neither theophylline (Figure 3.17) nor aminophylline caused any marked changes in blood pressure and the highest dose of adenosine studied (100 μg) did not alter arterial blood pressure when injected post-theophylline.

Imidazole

The effect of imidazole, a stimulator of phosphodiesterases (Butcher and Sutherland, 1962; Huang and Kemp, 1971) was also investigated on spontaneous chemoreceptor discharge. Imidazole (10 - 400 μg i.c.) was tested in two experiments. High doses (400 μg) inhibited markedly chemoreceptor discharge. Recordings from a single chemoreceptor unit showed a reduction from 0.48 ct/s to 0 ct/s in the 10 to 60 s that followed the injection of imidazole; the discharge recovered in the next 60 - 80 s. Lower doses (10 μg) increased transiently chemoreceptor discharge from 6.4 ct/s (recordings from 2 - 3 chemoreceptor units obtained in another experiment) to 8.4 ct/s in the 60 s that followed the injection of imidazole.

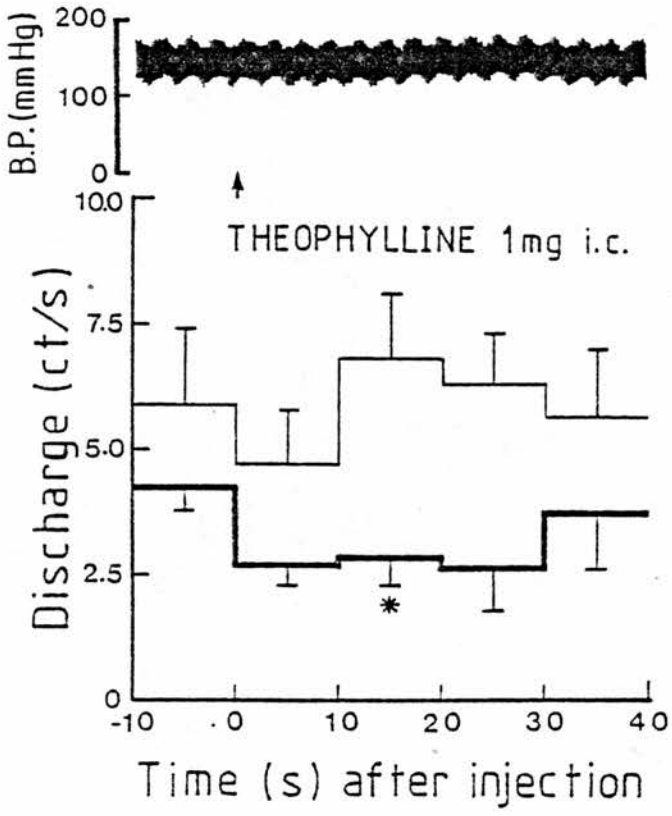


FIGURE 3.17

Effect on spontaneous chemoreceptor discharge (ct/s) of injecting theophylline 1 mg i.c. (▬) compared with that of injecting Locke solution (0.3 ml) (—) in five cats. Discharge was averaged over 10 s periods following the injection. The upper panel shows the effect on arterial blood pressure of injecting (arrow) 1 mg of theophylline in one experiment.

* $P < 0.05$ (paired t-test).

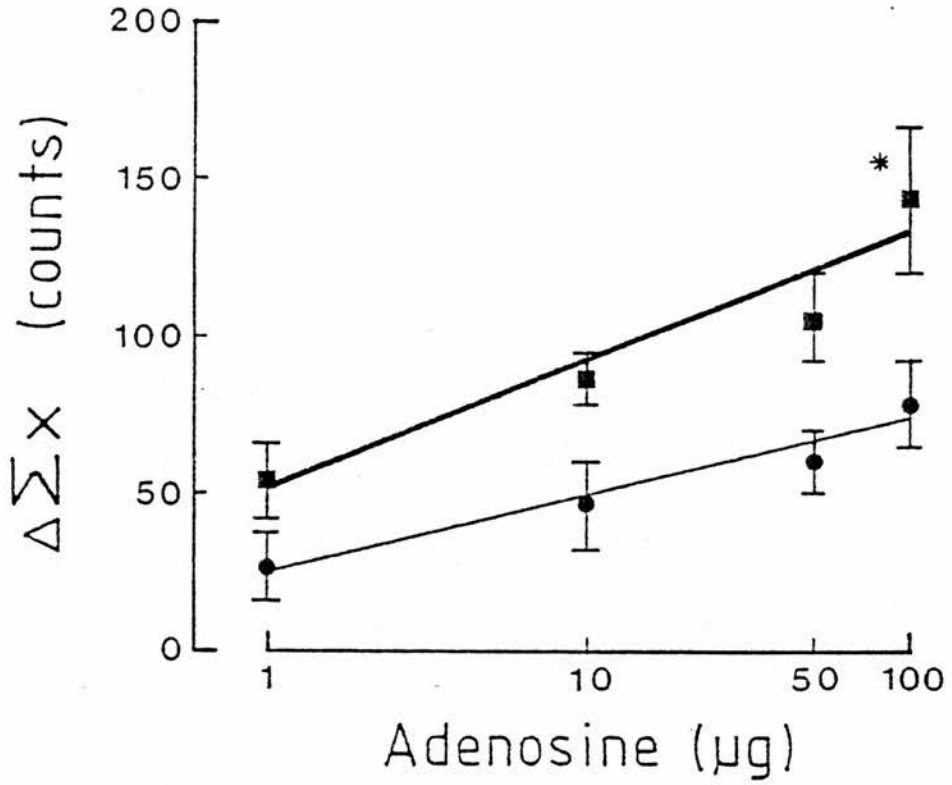


FIGURE 3.18

Dose-response data for adenosine obtained before (●) and after (■) theophylline (1 mg i.c.) in four experiments. Doses (μg i.c.) are plotted on a $\log_{10}$ scale and chemoreceptor responses expressed as the mean $\Delta\Sigma x$ for the four experiments ± s.e. mean. Lines were fitted to the data by the method of least squares. Averaged values ± s.e. mean (ct/s) for the pre-injection (15 s) control periods are as follows: 1 μg 4.5 ± 1.0 before theophylline, 6.5 ± 1.4 after; 10 μg 5.5 ± 1.4 before, 7.8 ± 1.7 after; 50 μg 3.5 ± 1.1 before, 5.2 ± 1.4 after; 100 μg 3.9 ± 1.7 before, 5.1 ± 2.0 after.

* $P < 0.05$ (paired t-test).

α-flupenthixol

The effects of adenosine on chemoreceptor discharge were studied in two cats before and after injecting α-flupenthixol (0.2 mg/kg i.c.), a dopamine antagonist. Figure 3.19 illustrates the chemoinhibitory responses to dopamine (5 μg i.c.) obtained before and after α-flupenthixol and the chemoexcitatory responses to adenosine (10 - 100 μg i.c.) observed before and after α-flupenthixol. As can be seen, α-flupenthixol antagonized the inhibition caused by dopamine but potentiated the chemoexcitatory responses induced by adenosine.

Cyclic AMP and dibutyryl cyclic AMP

Adenosine increases the concentration of cyclic AMP in many preparations both *in vitro* and *in vivo* (for a review see Daly, 1977). In order to know whether cyclic AMP and dibutyryl-cyclic AMP have any effect on arterial chemoreceptors, the substances were administered i.c. Figure 3.20 shows dose-response curves to both cyclic AMP (10 - 800 μg) and dibutyryl-cyclic AMP (10 - 800 μg) obtained in the same cat together with responses to adenosine (10 - 100 μg i.c.). As can be seen both cyclic AMP and dibutyryl-cyclic AMP increased spontaneous chemoreceptor discharge but both these substances were less effective than adenosine.

Adenosine analogues: N^6-methyladenosine, 3'-deoxyadenosine, 2'-chloroadenosine and 2'-deoxyadenosine

Chemoreceptor responses to adenosine analogues were compared with those to adenosine in four cats and the results are summarized in Figure 3.21. N^6-methyladenosine and 2'-chloroadenosine which act on R-site increased spontaneous chemoreceptor discharge, and

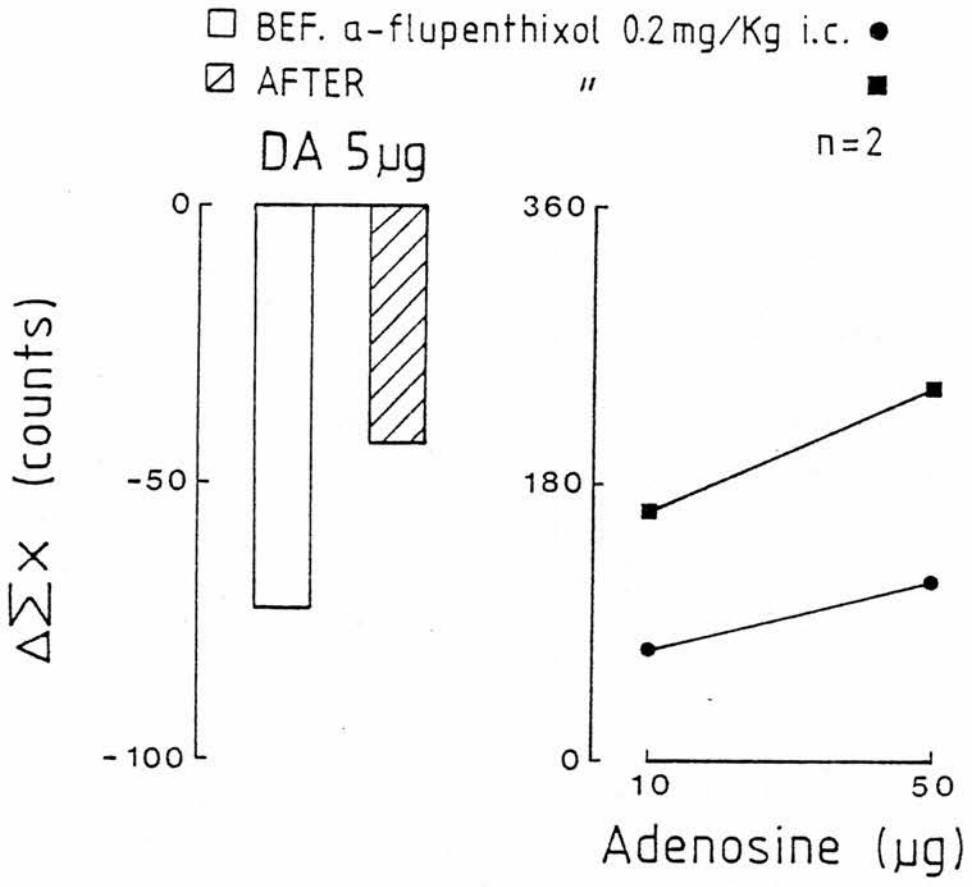


FIGURE 3.19

Effects on chemoreceptor discharge of injecting i.c. dopamine (DA) and adenosine before (BEF.) and after α-flupenthixol in two cats. Averaged values (ct/s) for the pre-injection (10 - 15 s) control periods are as follows: DA 10.2 before α-flupenthixol, 14.9 after α-flupenthixol; adenosine 10 µg 10.7 before, 18.9 after; 100 µg 10.7 before, 13.1 after.

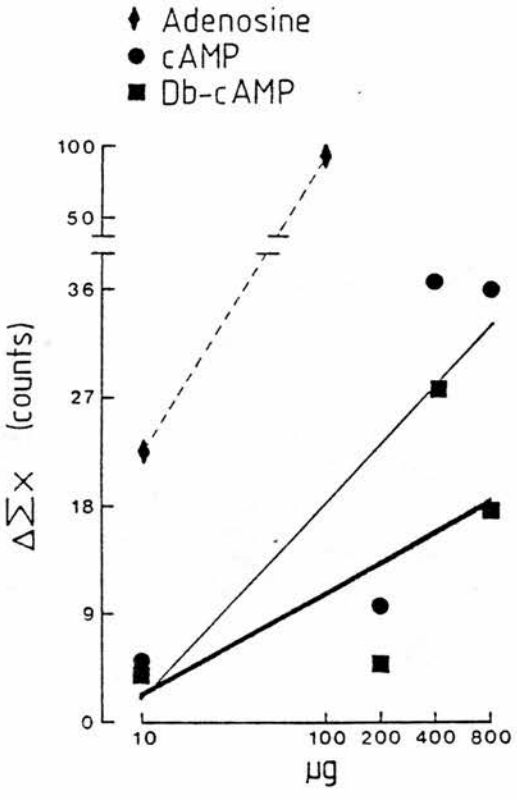


FIGURE 3.20

Effects of cyclic AMP (cAMP) and dibutyryl-cyclic AMP (Db-cAMP) on spontaneous chemoreceptor activity compared with adenosine. Recordings from a single chemoreceptor unit. Lines for cAMP and Db-cAMP were fitted to the data by the method of least squares. Values (ct/s) for the pre-injection (15 s) control periods are as follows: adenosine 10 µg 0.1, 100 µg 0.2; cAMP 10 µg 0.3, 200 µg 0.1, 400 µg 0.1, 800 µg 0.1; Db-cAMP 10 µg 0.1, 200 µg 0.1, 400 µg 0.3, 800 µg 0.1.

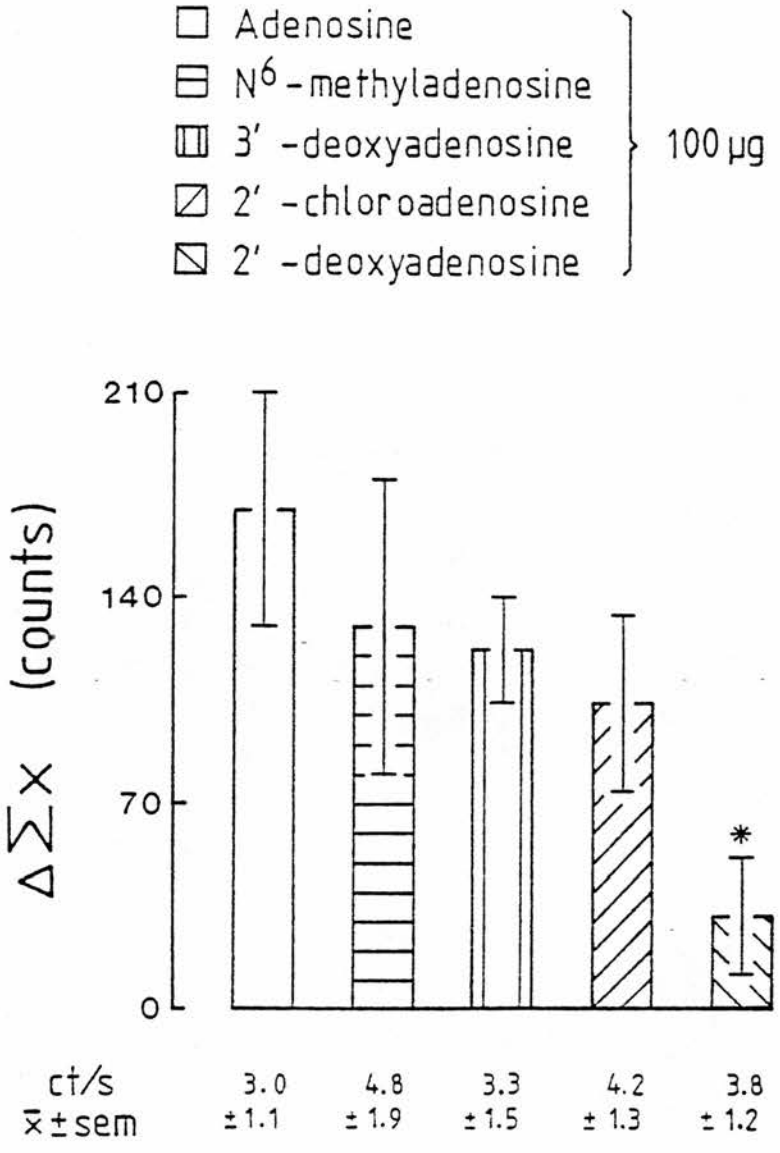


FIGURE 3.21

Effects of adenosine analogues on spontaneous chemoreceptor discharge and their comparison with adenosine in four cats. Data were pooled and shown as mean ± s.e. mean. At the bottom averaged values (ct/s) ± s.e. mean for the pre-injection (15 s) control periods are shown.

* P <0.05 (paired t-test compared with any of the other responses).

their effects were very similar to adenosine; 3'-deoxyadenosine which acts in both R and P-sites (Londos and Wolff, 1977) but has higher affinity for R-site also increased chemoreceptor discharge, whereas 2'-deoxyadenosine which acts only on P-site had little or no effect on spontaneous discharge.

Adenosine and hypoxia

It has been shown in a number of tissues (e.g. heart) that hypoxia can release considerable amounts of adenosine (see e.g. Berne, 1980). Two different experimental protocols were designed to test the effect of hypoxia in the presence of a high concentration of adenosine and the effect of adenosine in the presence of hypoxia on arterial chemoreceptor discharge. In the first experimental protocol after 2 min of infusing adenosine (100 µg/min i.c.) a hypoxic stimulus (10% O_2 + 90% N_2) was applied, and a marked increase in chemoreceptor discharge occurred additional to the increase caused by adenosine (see Figure 3.22). After withdrawing the hypoxic stimulus, chemoreceptor discharge returned to about the same level as before. This suggests that, if adenosine is involved in the hypoxic stimulation of the carotid body chemoreceptors, other substances besides adenosine are responsible for the chemoexcitation.

The second experimental protocol is illustrated in Figure 3.23. Adenosine (50 µg/min i.c.) was infused in the presence of hypoxia and as can be seen it still increased chemoreceptor activity. This seems to indicate that the adenosine receptor is not fully saturated by any adenosine that might be released during hypoxia.

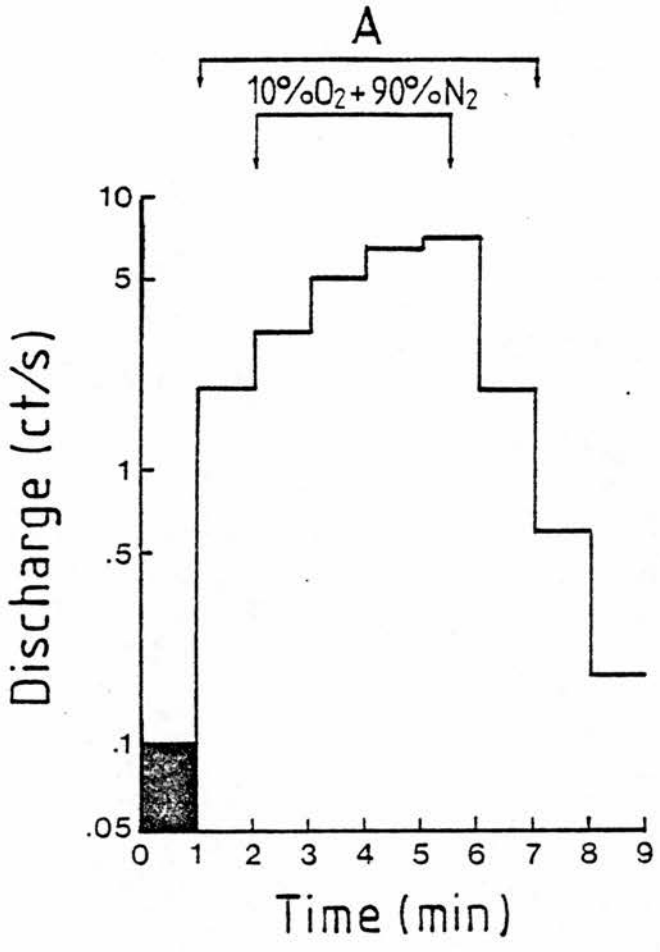


FIGURE 3.22

Effect of hypoxic stimulation (ventilation with 10% O_2 + 90% N_2) on chemoreceptor discharge (ct/s) in the presence of A) adenosine (100 µg/min i.c.) (recordings from a single chemoreceptor unit). The black rectangle represents the discharge averaged 60 s before starting infusion of adenosine.

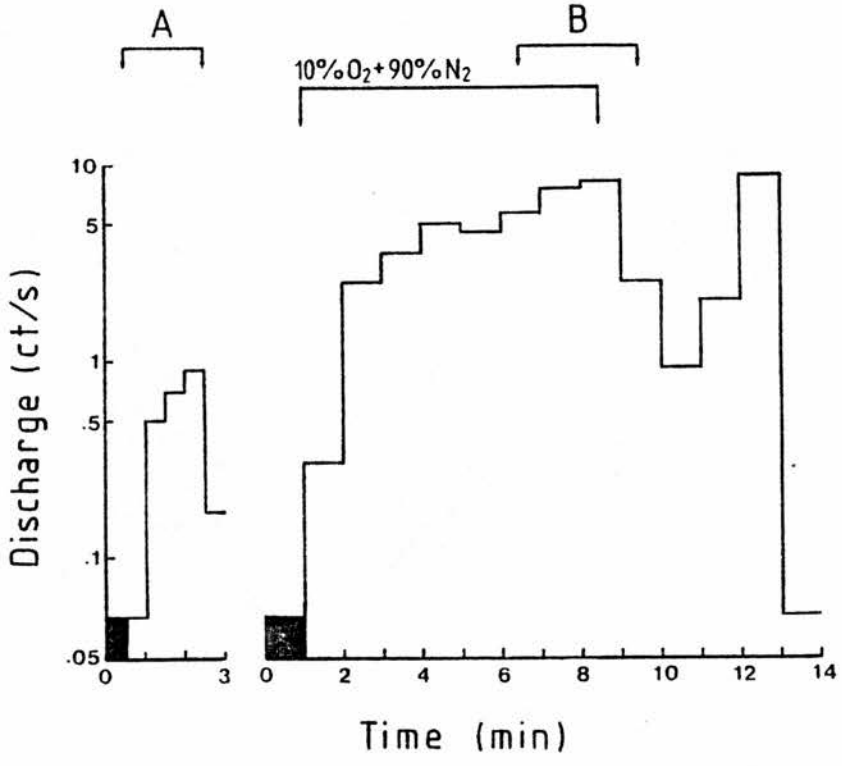


FIGURE 3.23

Effect of an adenosine infusion (50 µg/min) on chemoreceptor discharge (ct/s) before (A) and (B) in the presence of hypoxic stimulation (10% O_2 + 90% N_2). Recordings from a single chemoreceptor unit. The left side of the figure shows the response to an adenosine infusion recorded before hypoxic stimulation. The black rectangles represent the pre-infusion (control) discharge averaged in the 30 s (left) and 60 s (right) that preceded the adenosine infusion or the hypoxic stimulation.

DISCUSSION

The results of this section show that the predominant effect of injecting or infusing adenosine close-arterial to the cat carotid chemoreceptors is an increase in spontaneous chemosensory discharge frequency. Adenine nucleotides (AMP, ADP and ATP) injected under the same conditions and in equivalent amounts caused similar effects on spontaneous chemoreceptor discharge.

When ATP was injected in the presence of adenosine or adenosine in the presence of ATP, their chemoexcitatory actions are reduced, which can be taken to indicate that ATP and/or adenosine activate carotid body chemoreceptors through a mechanism similar to that used by adenosine and/or ATP. Further support for the idea that both substances act by a similar mechanism is that the effects of lower doses are potentiated, and those of higher doses antagonized by dipyridamole.

An issue of considerable debate has been whether prior degradation of the adenine nucleotides to adenosine is necessary before they can activate the receptor, or whether the receptor will accept a variety of different polyphosphate side chains on the 5' position. This problem was investigated with the methylene isosters of 5'-ATP, the α-β-methylene ATP and the β-γ-methylene ATP. The phosphorous methylene bond is considered to be stable to metabolic transformations involving either hydrolysis or phosphate transfer (Yount, 1975). The α-β-methylene ATP was found to decrease rather than increase chemoreceptor discharge and β-γ-methylene ATP increased chemoreceptor discharge, though less effectively than ATP. This suggests that even though the β,γ bond in the ATP analogue is stable, cleavage could still occur at the α,β position yielding the easily metabolized compound 5'-AMP. However, when the bond in the α,β

position is stabilized the compound does not increase chemoreceptor discharge, actually decreased discharge an effect associated with an increase in B.P. Similar types of activity for the ATP analogues in comparison with ATP were found in the brain (Phillis, Edstrom, Kostopoulos and Kirkpatrick, 1979). The results obtained with the ATP analogues taken together with the longer delay to onset of the chemoreceptor responses seen with ATP as compared with adenosine suggests that the ATP effect might depend on its previous hydrolysis to adenosine.

The increase caused by α-β-methylene ATP on B.P. suggests that ATP itself has a vasoconstrictor effect and this being consistent with a recent report by Chiba, Ohhashi and Azuma (1980). These authors described that in isolated fragments from the common carotid and internal carotid arteries of dogs, ATP has a potent vasoconstrictor effect.

As mentioned in Section I, General Introduction, a number of authors reported that ATP causes chemoreceptor activation (Jarisch, Landgren, Neil and Zotterman, 1952; Dontas, 1955; Anichkov and Belen'kii, 1963; Krylov and Anichkov, 1968; Roumy and Leitner, 1977). These findings were confirmed in the present work, and evidence was now produced suggesting that the chemoreceptor excitation by ATP depends on its previous hydrolysis to adenosine.

The present work also confirmed the results of Joels and Neil (1968) suggesting that cyclic AMP stimulates carotid body chemoreceptors. These results were obtained in vascularly isolated carotid bodies. In this preparation these authors described that ATP reduces chemoreceptor discharge evoked by continuous perfusion of NaCN or dinitrophenol whereas ADP and AMP had little or no effect. In the

present work ADP and AMP were as effective as ATP. Since effects of ATP on responses evoked by NaCN, CO_2, ACh or dopamine were not investigated it is not possible to compare the present results with those obtained by Joels and Neil (1968) in vascularly isolated carotid bodies.

The vascular effect

Adenosine can cause vasodilation in a number of mammalian organs, including the heart, skeletal muscle, brain (see e.g. Berne, Foley, Watkinson, Miller, Winn and Rubio, 1979) and we cannot, therefore, preclude the possibility that adenosine was changing blood flow in the carotid body and thereby, perhaps, altering chemoreceptor discharge, even though it had little overall effect on the systemic blood pressure. When the vasodilator effect of adenosine was potentiated by dipyridamole, a drug which potentiates the vasodilator properties of exogenous adenosine (Bretschneider, Bernard, Kochsiek and Scheler, 1959), a decrease rather than an increase in chemoreceptor discharge was observed; this is consistent with the effect of other potent vasodilators (e.g. sodium nitrite, sodium nitroprusside - Docherty, 1980) which also cause decreases in discharge following their intracarotid injections, albeit more delayed. Moreover, if chemoreceptor activation by adenosine has been a result of its vasodilator properties, the effect should have been blocked by theophylline (Berne, Rubio and Curnish, 1974; Wahl and Kuschinsky, 1976) rather than potentiated as was observed.

The increase in spontaneous chemoreceptor discharge by dipyridamole (i.c.) is compatible with a role for endogenous adenosine on carotid body chemoexcitation. Further support for such a role could be obtained by

using adenosine deaminase inhibitors such as deoxycoformycin which also potentiates the effects of endogenous adenosine in other tissues (e.g. central nervous system, see Phillis, Edstrom, Kostopoulos and Kirkpatrick, 1979).

The adenosine receptor

The receptor for adenosine appears to be specific since adenine, the purine nucleosides inosine and guanosine, and the pyrimidine nucleosides cytidine and uridine were virtually ineffective on the chemoreceptors.

The adenosine receptor seems to be externally located since the adenosine uptake blocker, dipyridamole, potentiated the chemoreceptor activation induced by adenosine. It is at present impossible to know whether the receptor is located on the sensory nerve endings, glomus type I and/or type II cells or, and indeed if adenosine receptors may be present, in all these structural components. The potentiation observed in the responses evoked by ACh and dopamine, which some authors used to consider as acting mainly on the sensory nerve endings (see Eyzaguirre and Monti-Bloch, 1980) could be construed as evidence that this structural component might be involved in the adenosine effect. Since the location of the ACh receptors in the carotid body structures remains to be established any conclusions of evoked responses have to be somewhat tentative (see Eyzaguirre and Monti-Bloch, 1980; Eyzaguirre and Fidone, 1980).

It is unlikely that the effect results from direct activation of the sensory nerve fibres because adenosine does not change either amplitude or duration of the compound action potential in the frog-sciatic nerve (Ribeiro and Dominguez, 1978) or of action potentials of unmyelinated fibres in the brain (Stone, 1980).

Direct activation of nerve endings by adenosine has been reported. For example, Siggins, Gruol, Padjen and Forman (1977) found that adenosine depolarizes neurones of explanted amphibian sympathetic ganglia, and Bleehen and Keele (1977) described algogenic actions of adenosine on the human blister base.

An adenylate cyclase-cyclic AMP system is apparently located in the sinus nerve endings (Fitzgerald, Rogus and Dehghani, 1977) and it may be that adenosine interacts with this in a manner similar to that described for the brain *in vivo* (e.g. Davies, Taylor, Gregson and Quinn, 1980). On the other hand both cyclic AMP and Db-cyclic AMP increased chemoreceptor activity. The effect of cyclic AMP might depend on its being hydrolysed to adenosine (Mah and Daly, 1976) as appears to happen with ATP in the present work. However, this is not probably the case for Db-cyclic AMP which has a high liposolubility (Henion, Sutherland and Posternak, 1967) and hence can easily cross cell membranes. Its small effect may reflect that the concentration used to modify intracellular concentration in the carotid body is too small and not steady. It would be interesting to apply it to an *in vitro* carotid body preparation.

In many preparations exogenous adenosine decreases release of neurotransmitters [e.g. ACh (Ginsborg and Hirst, 1972; Ribeiro and Walker, 1975; Vizi and Knoll, 1976; Gustafsson, Hedqvist, Fredholm and Lundgren, 1978); noradrenaline (Hedqvist and Fredholm, 1976; Verhaeghe, Vanhoutte and Shepherd, 1977; Wakade and Wakade, 1978); dopamine (Michaelis, Michaelis and Myers, 1979); γ-aminobutyric acid (Hollins and Stone, 1980b)]. It is perhaps not too surprising that adenosine decreases the output of substances which are released in a calcium-dependent manner, because adenosine depresses uptake of

calcium by synaptosomes depolarized by potassium (Ribeiro, Sá-Almeida and Namorado, 1979). If adenosine exerts a similar action in the carotid body, its excitatory effect could be interpreted as being the consequence of decreased release of an inhibitory transmitter and/or modulator. Dopamine (Chiocchio, Biscardi and Tramezzani, 1966; Zapata, Hess, Bliss and Eyzaguirre, 1969) and the enkephalins (met- and leu-ENK) (Lundberg, Hökfelt, Fahrenkrug, Nilsson and Terenius, 1979; Wharton, Polak, Pearse, McGregor, Bryant, Bloom, Emson, Bisgard and Will, 1980) appear to be present in the cat carotid body and both exogenous dopamine (Zapata, 1975; Docherty and McQueen, 1978) and the enkephalins (see Section V) depress spontaneous chemoreceptor activity. Osborne and Butler (1975) suggested that tonically released dopamine may suppress chemoreceptor discharge, and although evidence which is not in agreement with their hypothesis has been obtained (e.g. Docherty and McQueen, 1978), the present effect of adenosine could be explained in terms of adenosine inhibiting the release of dopamine, or some other substance which is tonically active in inhibiting discharge (? enkephalins). In support of a decrease on the release of dopamine by adenosine are the present results in which α-flupenthixol potentiated the chemoexcitatory responses to adenosine.

Adenosine does not operate through a theophylline-sensitive receptor since theophylline in fact potentiated rather than reduced responses to adenosine.

Theophylline appears unable to prevent activation of P_2-purinergic receptors. These receptors, as so far described, seem to be localized post-junctionally (see Burnstock, 1978). De Mey, Burnstock and Vanhoutte (1979) found that in the canine saphenous vein theophylline antagonizes the inhibitory effect of ATP on neurogenic responses, but

not its direct contractile effect. According to these authors this suggests the presence of both inhibitory pre-synaptic (P_1) and excitatory post-synaptic (P_2) receptors. In the present study the excitatory effect of adenosine was unaffected by low doses of theophylline (0.1 - 0.2 mg) but potentiated by a slightly higher dose (1 mg), a finding which is compatible with a P_2-excitatory post-junctional effect. However, the potentiation might also have resulted from theophylline antagonizing a chemodepressant component of the adenosine response, which might normally be masked by the excitatory component. Such a chemodepressant component would result from adenosine decreasing a tonically released excitatory transmitter - e.g. ACh (see Osborne and Butler, 1975) or another excitatory influence which cannot be antagonized by theophylline. In the brain adenosine decreases the release of dopamine through a theophylline-insensitive receptor (Michaelis, Michaelis and Myers, 1979). Other possibilities to explain the potentiation are: (1) that both adenosine and theophylline have in common the ability to increase the cyclic AMP concentration, e.g. theophylline by inhibiting phosphodiesterases (e.g. Butcher and Sutherland, 1962), adenosine by activating the adenylate cyclase (see Daly, 1977); and (2) the potentiation might result from theophylline inhibiting adenosine uptake as has been observed in rat brain synaptosomes (Bender, Wu and Phillis, 1981).

Other classifications of adenosine receptors have been proposed. Londos and Wolff (1977) postulated the existence of an adenosine receptor with a P-site internally located and particularly sensitive to 2'-deoxyadenosine (a P-site agonist) and a R-site externally located and predominantly sensitive to R-site agonists such as N^6-methyl-adenosine and 2'-chloroadenosine. Substances such as 3'-deoxyadenosine

act in both R- and P-sites but has a higher affinity for R-site. The present results are compatible with a R-site adenosine receptor in carotid body since only R-site agonists (N^6-methyladenosine or 2'-chloroadenosine) or with higher affinity for R-site (3'-deoxyadenosine) were effective. According to the same authors the P-site would be responsible for inhibition of adenylate cyclase and consequent decrease in cyclic AMP whereas R-sites would be associated with stimulation of adenylate cyclase and subsequent increase in cyclic AMP.

In summary, adenosine and adenine nucleotides increase chemoreceptor discharge. The effect of adenine nucleotides seems to depend on their previous hydrolysis to adenosine. Adenosine appears to operate through a specific adenosine receptor, externally located, theophylline-insensitive and the R-site kind of receptor.

SECTION IV

The Effects of Peptides

INTRODUCTION

Evidence has been produced showing that neuropeptides such as met-ENK, leu-ENK, SP and VIP, or closely related immunoreactive substances are present in the cat carotid body (Lundberg, Hökfelt, Fahrenkrug, Nilsson and Terenius, 1979; Cuello and McQueen, 1980; Wharton, Polak, Pearse, McGregor, Bryant, Bloom, Emson, Bisgard and Will, 1980; Fitzgerald, Raff, Garger, Fechter, Anand and Said, 1981).

It has also been shown that met-ENK (McQueen, 1981), SP (McQueen, 1980) and VIP (Fitzgerald, Raff, Garger, Fechter, Anand and Said, 1981) can affect spontaneous chemoreceptor discharge when injected close-arterial to the cat carotid body. The opportunity was taken to investigate further the action of various polypeptides, including some of those identified as being present in the carotid body on the cat carotid chemoreceptors. During studies on the enkephalins the effects on morphine were also investigated in order to have some insight about the receptor involved in the chemosensory action of opiates.

RESULTS

Methionine-Enkephalin

As illustrated in Figure 4.1, met-ENK caused a dose-related decrease in spontaneous chemoreceptor discharge with a rapid onset, the effect commencing within 1 to 2 s of beginning the injection. The inhibition was followed by a gradual return, reaching control (pre-injection) levels within 30 - 45 s following low doses of met-ENK, but taking up to 5 min to recover after high doses. The inhibitory effect was consistent, as can be gauged from the standard errors

(Figure 4.1), and there was no evidence of tachyphylaxis occurring when doses were administered at 7 min interval. Intravenous injections of met-ENK (10 - 100 μg) also inhibited chemoreceptor discharge, although to a lesser extent and after a longer delay than the same doses by intracarotid injection.

Low doses of met-ENK had little or no effect on arterial blood pressure, whereas higher doses (i.c. or i.v.) caused a fall in B.P. (see Figure 4.1).

Leucine-Enkephalin

The effect of leu-ENK on the spontaneous chemoreceptor discharge is illustrated in Figure 4.2. The discharge was averaged in 15 s periods and the response was followed over a 90 s post-injection period. As can be seen leu-ENK (0.1 - 100 μg i.c.) caused a dose-dependent decrease in spontaneous chemoreceptor discharge with a rapid onset.

As with met-ENK, no tachyphylaxis was observed and low doses of leu-ENK had no appreciable effect on B.P.

Comparison of the depressant effects of methionine-, and leucine-enkephalin

Comparison between the depressant effects of met-ENK and leu-ENK was carried out in two cats. Figure 4.3 shows dose response curves (0.1 - 100 μg i.c.) obtained for both substances in the same animals. The inhibitory effects on spontaneous chemoreceptor discharge caused by both substances were similar when the substances were administered in the same doses. As the molecular weights of both met-ENK (m.w. $\simeq$ 556) and leu-ENK (m.w. $\simeq$ 574) are of the same order, the substances seem to be equipotent.

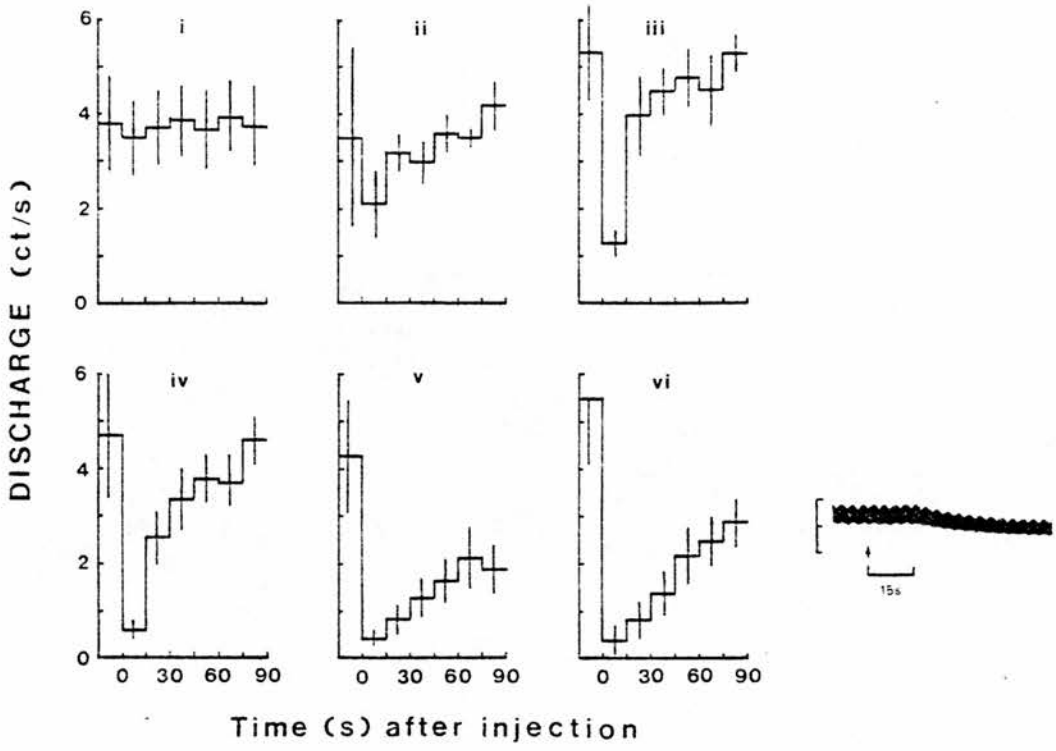


FIGURE 4.1

Effects on spontaneous chemoreceptor discharge (ct/s) of injecting Locke solution (0.3 ml i.c.) n=5 (i) and met-ENK 0.01 µg n=2 (ii), 0.1 µg n=5 (iii), 1 µg n=5 (iv), 10 µg n=5 (v), 100 µg n=5 (vi). Discharge was averaged over 15 s periods following the injection. Data from n experiments were pooled and are shown as the mean ± s.e. mean. The panel on the right shows an arterial blood pressure trace after injecting (arrow) 100 µg of met-ENK in one experiment. B.P. calibration, 0-100-200 mmHg.

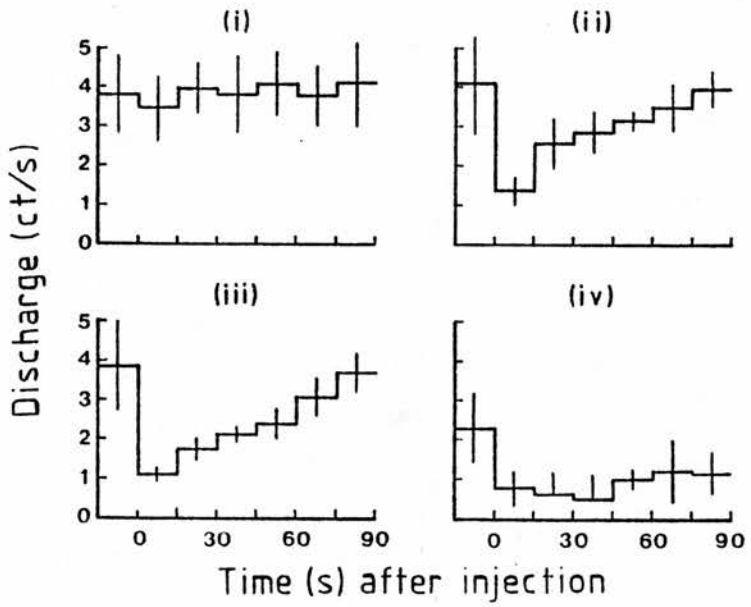


FIGURE 4.2

Effects on spontaneous chemoreceptor discharge (ct/s) of injecting (i.c.) Locke solution (0.3 ml) n=4 (i) and leu-ENK 1 μg n=3 (ii); 10 μg n=4 (iii); 100 μg n=2 (iv). Discharge was averaged over 15 s periods following the injection. Data from n experiments were pooled and are shown as the mean ± s.e. mean.

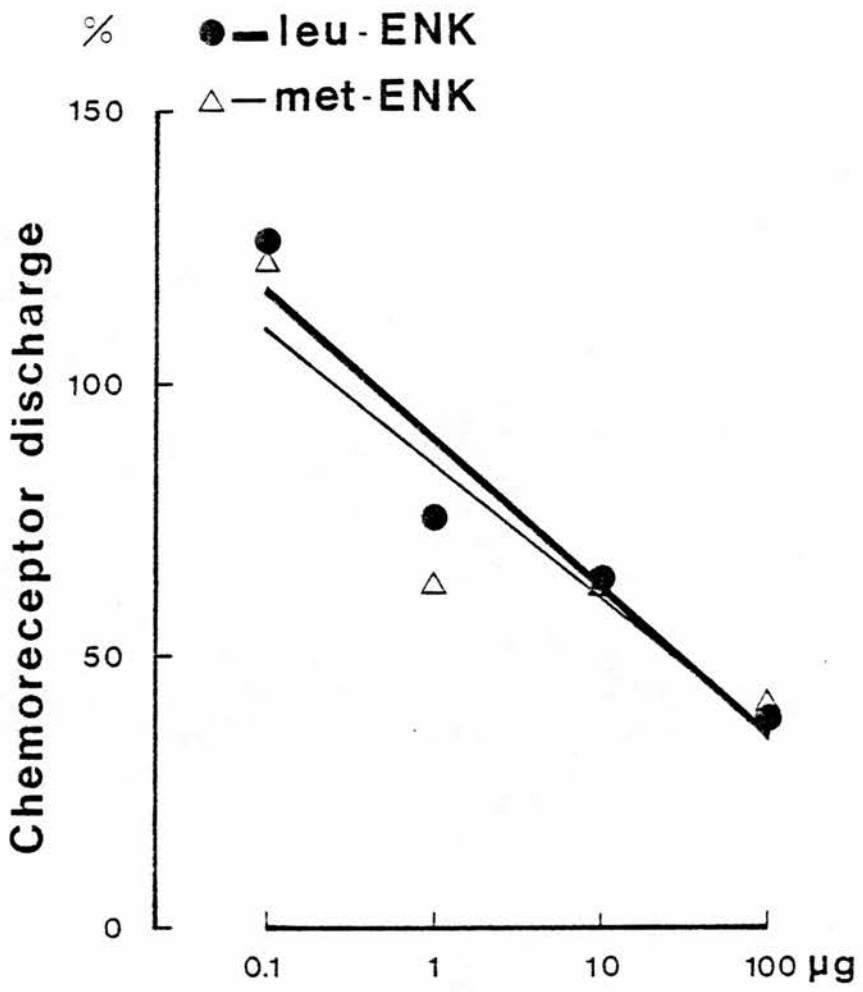


FIGURE 4.3

Chemoreceptor responses to doses of met-ENK in comparison with those to leu-ENK. Each point is the average from two experiments. Straight lines were fitted to the data by the method of least squares. Averaged values (ct/s) for the control (100%) periods are as follows: met-ENK 0.1 µg 1.5; 1 µg 2.2; 10 µg 4.4; 100 µg 2.7; leu-ENK 0.1 µg 2.7; 1 µg 8.2; 10 µg 7.9; 100 µg 3.2.

β-endorphin

The effects of β-endorphin on chemoreceptor activity were studied in three experiments. There was no appreciable difference between the actions of human and porcine β-endorphin, so, results have been expressed simply as responses to β-endorphin.

Injections of β-endorphin (0.1 - 50 μg i.c.) caused a biphasic response on spontaneous chemoreceptor discharge, an increase followed by a decrease (see Figure 4.5). The response lasted for about 3 min following the highest dose studied (50 μg) and was associated with a very slight fall in systemic blood pressure (Figure 4.4). Discharge was averaged over the 60 s period immediately following β-endorphin injection and compared with the averaged pre-injection (control) discharge (Figure 4.5). The results showed that the decrease in discharge was variable and from consideration of discharge during the second min following the injection (Figure 4.5) fairly long-lasting.

In one experiment, β-endorphin was also infused (3 μg/min i.c.) for 5 - 10 min but only slight changes were detected in spontaneous chemoreceptor discharge and B.P. Blood gas tensions and pH were not modified by the infusion.

Morphine

The overall effect of morphine on spontaneous chemoreceptor discharge was inhibitory, although the lowest dose studied, 0.1 μg, caused a slight increase in discharge (see Figure 4.6). Higher doses (100 μg) were associated with an inhibition of discharge which began within 1 to 2 s of starting the injection (Figure 4.6) and was dose-related.

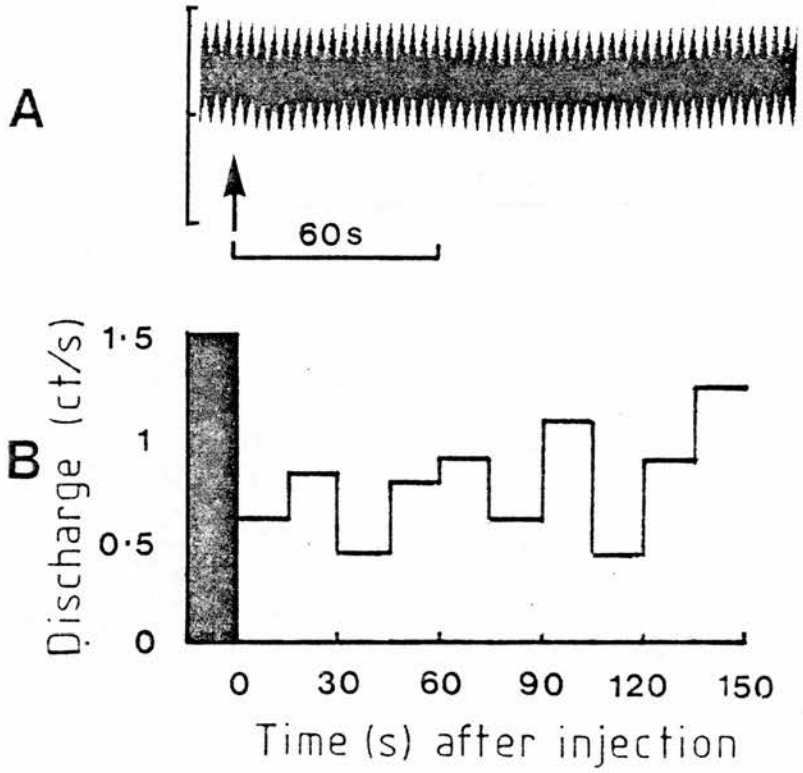


FIGURE 4.4

Effect of injecting (arrow) β-endorphin (50 μg i.c.) on: A) systemic arterial blood pressure; B) spontaneous chemoreceptor discharge averaged over 15 s periods. Recordings obtained from a single chemoreceptor unit. The black rectangle represents the discharge averaged in the 30 s that preceded injection. B.P. calibration is 0-100-200 mmHg.

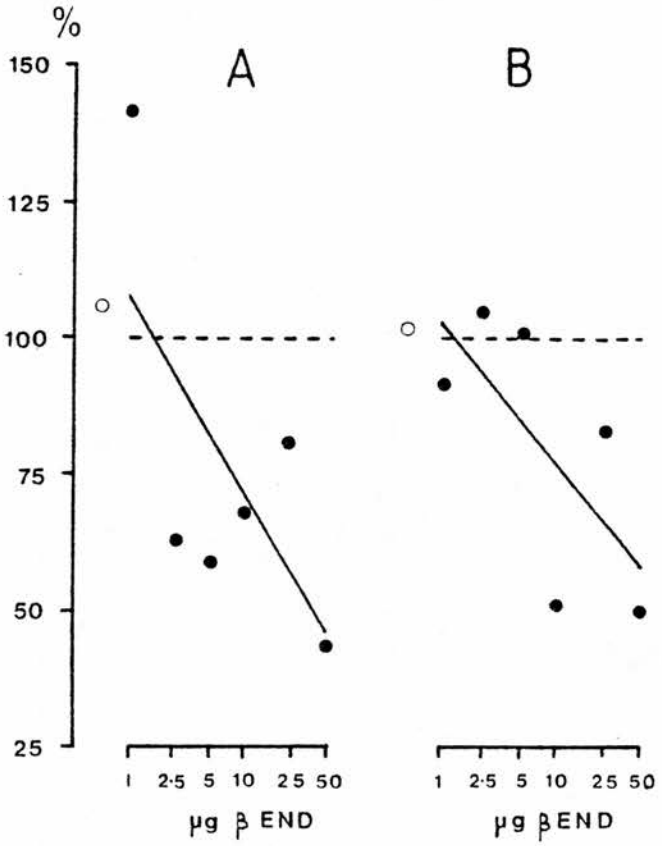


FIGURE 4.5

Effect of β-endorphin on spontaneous chemoreceptor discharge averaged over the first (A) or second (B) min following β-endorphin injection. Discharge was expressed as a percentage of the pre-injection frequency and plotted against dose of β-endorphin ($\log_{10}$ scale), straight lines being fitted to the responses (filled circles) by the method of least squares. Open circles illustrate the effect of injecting the drug vehicle (0.5% bovine serum albumin); the pre-injection (control) discharge frequency (100% = 1.5 ct/s) was determined by averaging spontaneous discharge during the 30 - 60 s immediately preceding each injection, and is represented. Recordings from a single chemoreceptor unit.

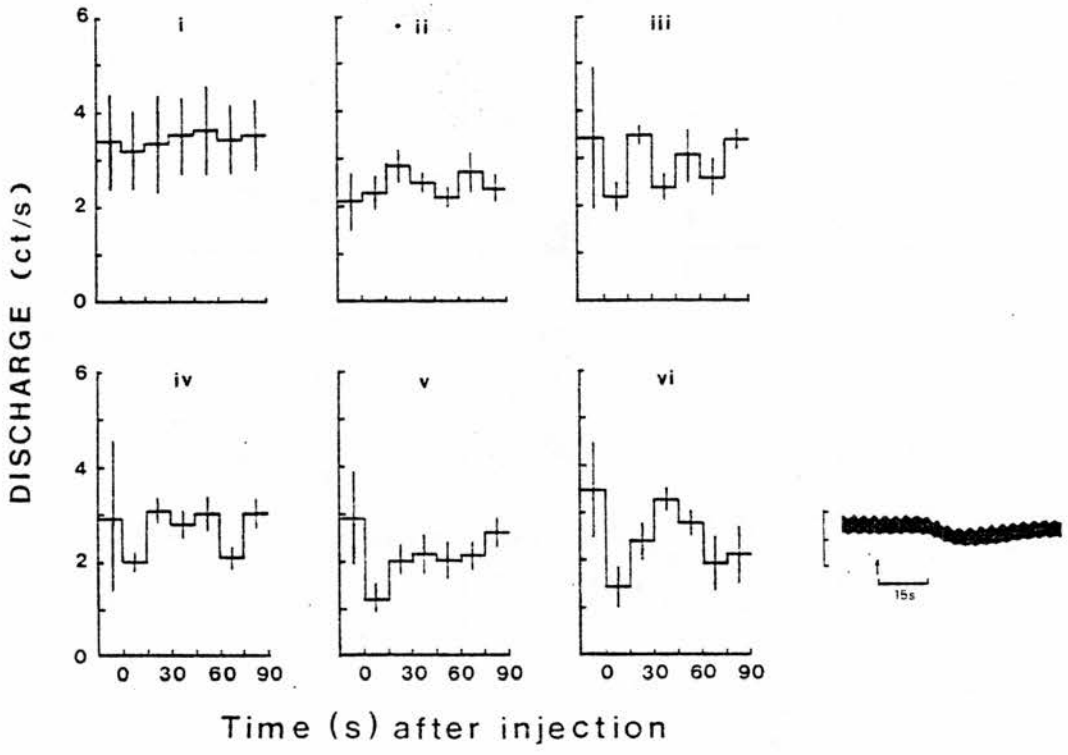


FIGURE 4.6

Effects on spontaneous chemoreceptor discharge (ct/s) of injecting i.c. Locke solution (0.3 ml) n=4 (i), and morphine 0.1 µg n=4 (ii); 1 µg n=3 (iii); 10 µg n=3 (iv); 100 µg n=4 (v); 1000 µg n=3 (vi). Discharge was averaged over 15 s periods following the injection. Data from n experiments were pooled and are shown as the mean ± s.e. mean. The panel on the right shows an arterial blood pressure trace after injecting (arrow) 1000 µg of morphine in one experiment. B.P. calibration, 0-100-200 mmHg.

Responses to morphine were rather variable, as can be seen from the standard errors, but there was no evidence of tachyphylaxis and responses to a low dose of morphine injected before and after the highest dose were very similar. The higher doses of morphine caused a fall in systemic blood pressure (B.P.) (see Figure 4.6). The overall effect was similar to that evoked by β-endorphin (see above).

Naloxone

Naloxone (0.2 mg i.c.) caused a slight increase in chemoreceptor discharge (Figure 4.7) and, after a delay of 15 to 45 s, a rise in B.P. lasting for at least 30 min. Additional doses of naloxone (0.4, 0.8 and 1.6 mg i.c. at 7 min intervals) had no further effect on chemoreceptor discharge or B.P.

When naloxone (0.2 mg i.c.) was injected during experiments in which met-ENK and/or morphine had previously been administered, there was also an increase, albeit somewhat variable, in spontaneous chemoreceptor discharge (Figure 4.7) and a rise in B.P.

Responses to met-ENK (1 μg i.c.), leu-ENK (1 μg i.c.) and β-endorphin (10 μg i.c.) obtained before and after injecting naloxone (0.4 mg i.c.) are shown in Figure 4.8. As can be seen the responses were attenuated after naloxone. Further reduction in the inhibition of the opiates was obtained when the substances were injected 5 to 20 min after injecting naloxone (5 mg i.v.).

Quantitative data confirmed these observations and are shown for β-endorphin in Figure 4.9, for leu-ENK in Figure 4.10 and for met-ENK in Figure 4.11.

In order to characterize the opiate receptor, more detailed analysis was carried out for data obtained before and after naloxone from met-ENK and morphine experiments.

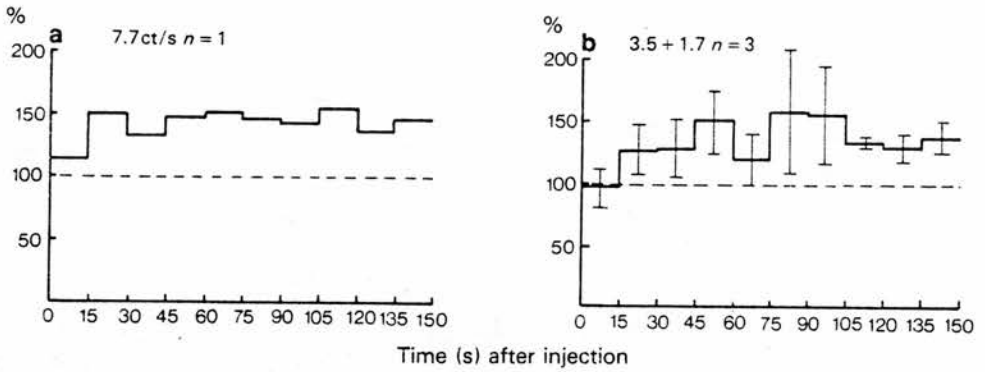


FIGURE 4.7

a . Illustrates the effect of naloxone (0.2 mg i.c.) on spontaneous chemoreceptor discharge in an experiment. b. Is the averaged spontaneous chemoreceptor discharge from three experiments in which the same dose of naloxone, 0.2 mg i.c., was injected following prior administration of met-ENK and/or morphine. Discharge was averaged over 15 s periods following the injection and expressed as the percentage of the averaged discharge ± s.e. mean in the 15 s pre-injection control period. Averaged values (ct/s) ± s.e. mean for the control 100% period is given.

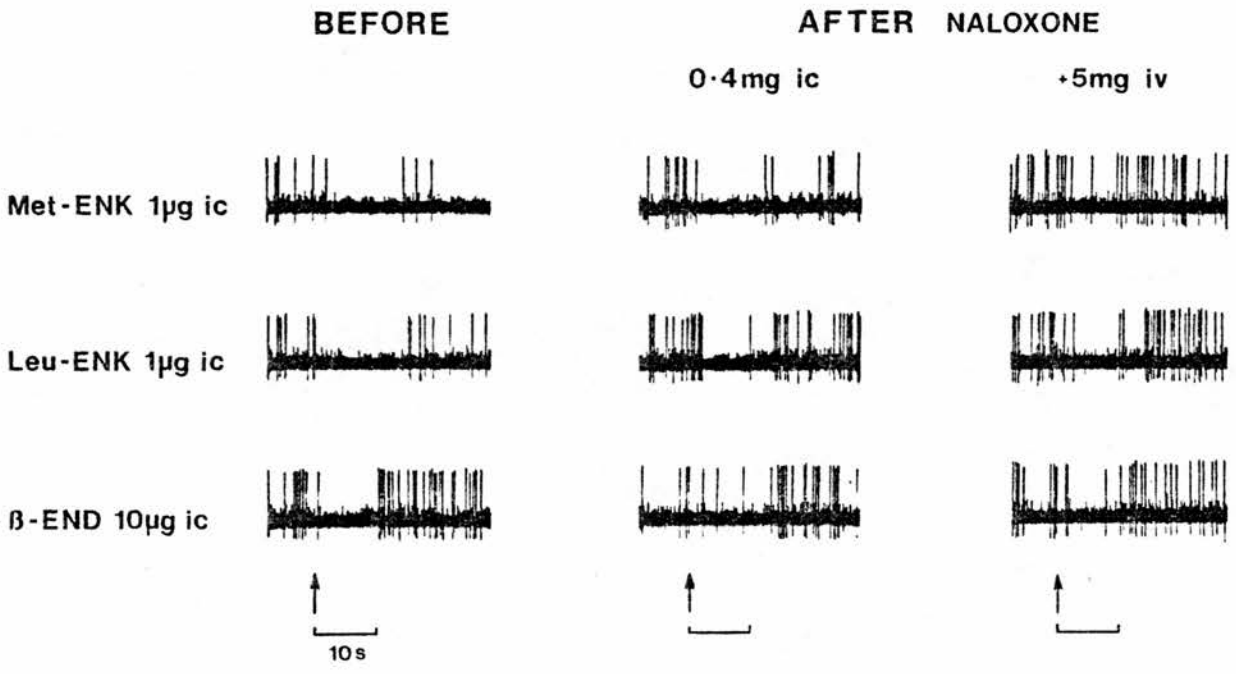


FIGURE 4.8

Responses to met-ENK, leu-ENK and β-endorphin (β-END) obtained before and after injecting naloxone 0.4 mg i.c. and again after injecting naloxone 5 mg i.v. in one cat. Neurograms of a single chemoreceptor unit.

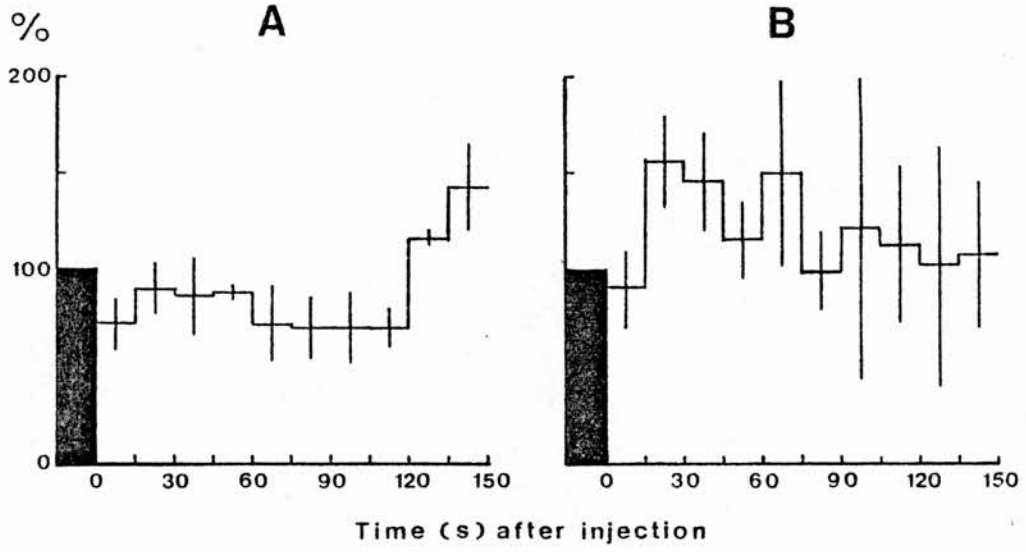


FIGURE 4.9

Chemoreceptor discharge following injection of β-endorphin 10 μg i.c. A) before and B) 5 min after naloxone (0.4 mg i.c.). Pooled data from three experiments, discharge expressed as a percentage of the pre-injection frequencies and shown as the average ± s.e. of mean in 15 s intervals during the 150 s post-injection period. The averaged control values were 4.8 ± 2.0 ct/s before and 2.7 ± 1.6 ct/s after naloxone.

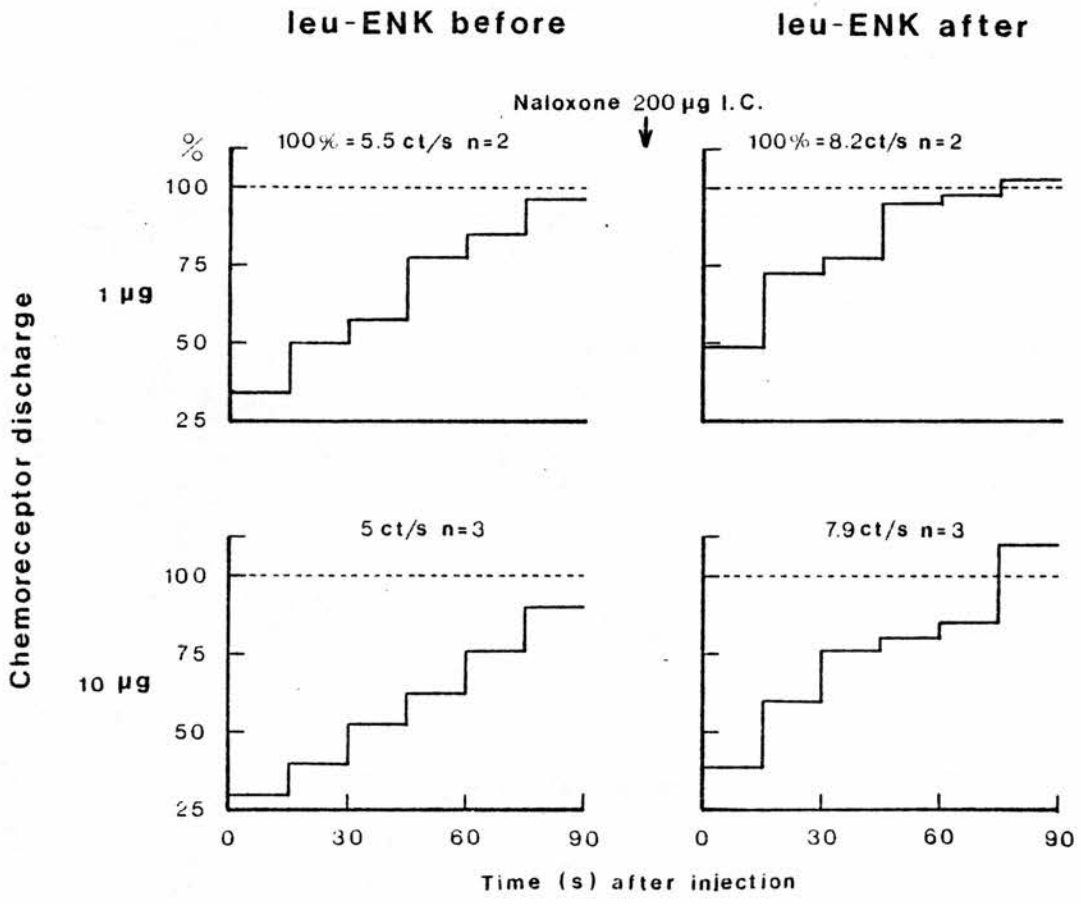


FIGURE 4.10

Effects of leu-ENK before and after naloxone (200 µg i.c.) on spontaneous chemoreceptor discharge. Pooled data from n experiments, discharge expressed as a percentage of the pre-injection frequencies is shown as the mean in 15 s intervals during the 90 s post-injection period. Averaged pre-injection (control) values are given.

Comparison of responses to met-ENK and to morphine obtained before and after injecting naloxone (0.2 mg i.c.) showed that, apart from a slight initial inhibitory effect, the inhibitory action of morphine was virtually abolished, and there was evidence of an overall increase in discharge. The chemoinhibitory effect of met-ENK was reduced by naloxone (see Figure 4.11).

Dose-response data were obtained by expressing the number of impulses in the post-injection period as a percentage of the number of impulses which would have been expected to occur in the same period had the pre-injection control discharge (averaged over 15 to 30 s) continued unaltered. It can be seen from Figure 4.11 that naloxone antagonizes the responses to met-ENK both for 60 s and 150 s post-injection periods. After intracarotid injection of naloxone (0.2 mg) morphine tended to increase discharge over these periods, this effect being inversely related to dose.

Evoked responses

Injections (i.c.) of ACh (50 µg), CO_2-equilibrated Locke solution (0.3 ml), NaCN (5 µg) and dopamine (5 µg) were made before and 5 to 20 min after a series of injections of either met-ENK or morphine, and during infusions of met-ENK (50 µg/min) and β-endorphin (5 µg/min). The results obtained before, during and after morphine, met-ENK and β-endorphin are summarized in Table 4.1. As can be seen the stimulant effects of ACh, CO_2 and NaCN were slightly and somewhat variably reduced after met-ENK whereas the inhibitory effect of dopamine was potentiated. Following morphine administration, responses to ACh and NaCN were reduced slightly, whereas those to CO_2 and dopamine were potentiated.

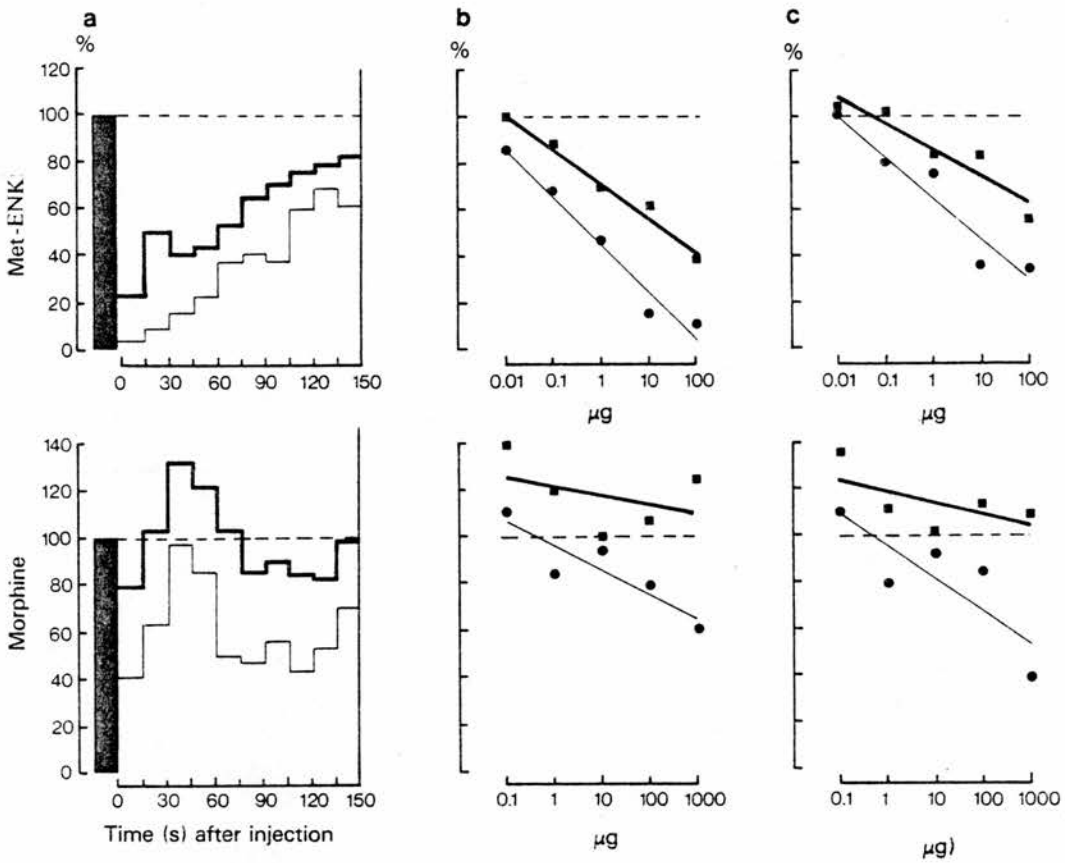


FIGURE 4.11

a) Pooled data showing the effects of met-ENK 100 μg (n=3) and morphine 1000 μg (n=2) on spontaneous chemoreceptor discharge before (—) and after (▬) injecting naloxone 0.2 mg i.c. Black rectangles represent the control discharge (100%), values for met-ENK being 3.7 ± 1.8 ct/s before and 3.1 ± 0.9 ct/s after naloxone, the corresponding values for morphine being 3.7 ± 1.7 and 4.9 ± 2.4 ct/s. b) is a plot of the total discharge over the 60 s post-injection period, expressed as a percentage of the total discharge which would have occurred in the same period if control discharge had continued unaltered (100% - dotted line), against $\log_{10}$ dose of met-ENK (pooled data from three experiments) or morphine (data from a single experiment) before (—) and after (▬) naloxone 0.2 mg i.c. Lines were fitted by the method of least squares. c) is similar to b) but the plot is of total discharge in the 150 s post-injection period and this includes the delayed inhibition seen with morphine.

Responses were also obtained before and during infusions of met-ENK (50 μg/min i.c.) and β-endorphin (5 μg/min i.c.), the dose of met-ENK being sufficient to inhibit spontaneous discharge throughout the infusion period. The effects of ACh and CO_2 were slightly potentiated whereas the response to NaCN was slightly reduced (Table 4.1). Since spontaneous discharge was suppressed it was not possible, under these conditions, to investigate the effect of met-ENK on the inhibitory response evoked by dopamine. The results obtained before, during and after an infusion of β-endorphin are also shown in Table 4.1. It was found that responses to the stimulants were reduced by β-endorphin in the concentration studied whereas the inhibitory effect of dopamine was potentiated. The influence of β-endorphin on responses evoked by dopamine and, to a lesser extent NaCN, was still evident 15 min after infusion had finished.

In a separate experiment the influence of β-endorphin (5 μg/min) on the inhibition evoked by an injection of met-ENK (10 μg i.c.) was studied. The effect of met-ENK was found to be very slightly enhanced (discharge, averaged over 150 s after the met-ENK injection and expressed as a percentage of the pre-injection discharge frequency [100%], was 71% before the infusion, 64% during the infusion and 73% 15 min after the infusion of β-endorphin).

Responses to ACh, NaCN, CO_2 and DA were not appreciably affected by naloxone (0.2 mg i.c.).

Methionine-enkephalin and substance P

It has been shown that SP influences chemoreceptor discharge (McQueen, 1980) and the present experiment was carried out to determine whether any interaction occurs on simultaneous infusion of

TABLE 4.1: Effects of morphine, methionine-enkephalin (met-ENK) and β-endorphin on evoked responses

Intracarotid injection of:	ACh (50 μg)	CO_2-Locke (0.3 ml)	NaCN (5 μg)	Dopamine (5 μg)	n=
A. Following morphine injections	83 ± 19	134 ± 11	89 ± 1	159 ± 61	3
B. Following met-ENK injections	77 ± 16	72 ± 17	63 ± 10	167 ± 24	5
C. During met-ENK infusions (50 μg min^{-1} i.c.)	137 ± 23	122 ± 9	84 ± 23	-	2
D. During an infusion of β-endorphin (5 μg min^{-1} i.c.)	85	52	48	190	1

Pooled data from n experiments showing chemoreceptor responses ($\Delta\Sigma\chi$), A following injections of morphine and B following injections of met-ENK. The results are expressed as mean percentages ± s.e. mean of the responses to the same doses administered before morphine or met-ENK (i.e. pre-injection response = 100%).

Injections were also made during infusions of met-ENK and β-endorphin and responses evoked compared with pre-infusion values (C and D).

met-ENK and SP close-arterial to the carotid body. The results obtained are shown in Figure 4.12. Locke solution infusions (0.1 ml/min) caused small oscillations (slight increases and decreases) in spontaneous chemoreceptor discharge, SP had a biphasic action (decrease followed by increase) and met-ENK reduced discharge, an effect which was most pronounced in the early part of the response; when the met-ENK infusion was stopped discharge increased above pre-infusion control levels. The delayed increase in discharge seen with SP was accompanied by a rise in end-tidal CO_2; there was no change in end-tidal CO_2 during or after met-ENK infusion.

SP and met-ENK were infused concurrently in equimolar concentrations, it being so arranged that met-ENK began to act before SP reached the carotid artery (see Figure 4.12). The pattern of response was very similar to that obtained with met-ENK alone, except that no post-infusion overshoot occurred. There was a rise in end-tidal CO_2 towards the end of the infusion. Both peptides caused hypotension, and when given together the overall effect on B.P. was approximately additive.

The inhibitory effect of opiates on carotid body chemoreceptors is not mediated by adenosine

It has been suggested that the inhibition of neurotransmitter release produced by opiates may be mediated by the initial release of adenosine (e.g. Stone and Perkins, 1979). The idea was advanced on the grounds that adenosine mimicks the effect of morphine, and theophylline antagonizes the actions of both adenosine and morphine. It was decided, therefore, to investigate whether theophylline and naloxone influenced the responses to opiates and adenosine respectively,

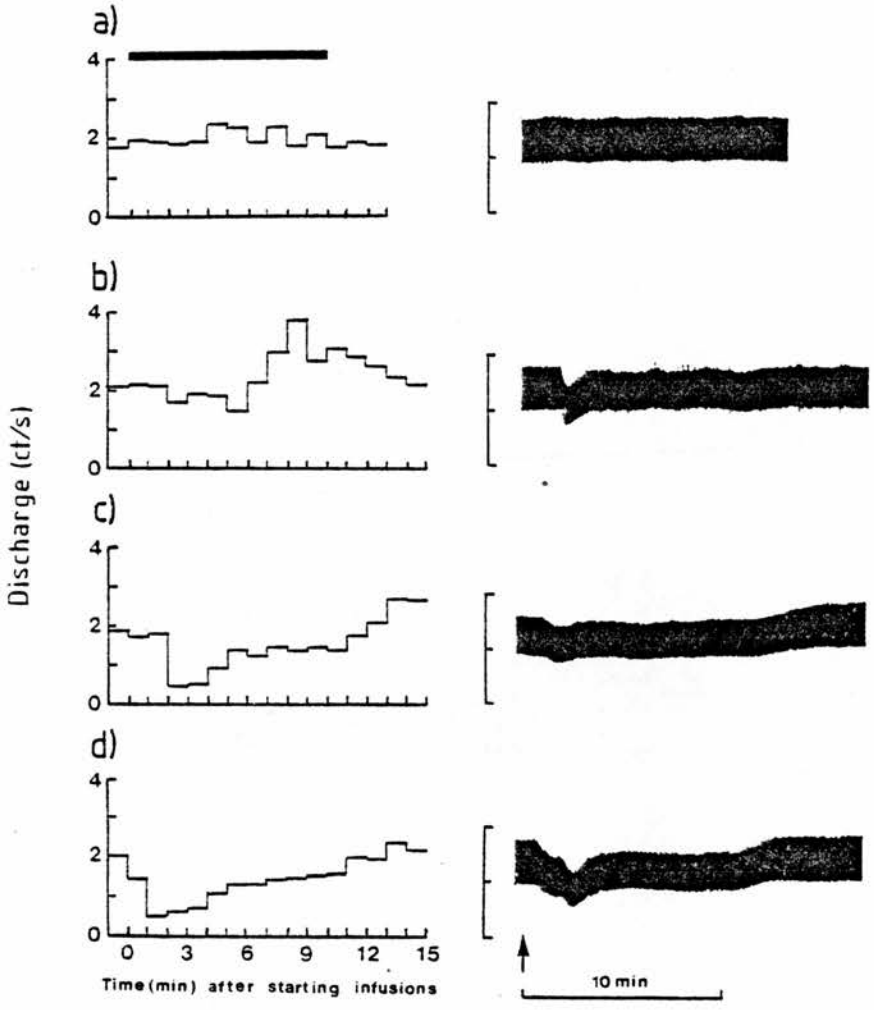


FIGURE 4.12

The left-hand side of the figure shows chemoreceptor discharge (ct/s) averaged over 60 s periods after starting intracarotid infusions (horizontal bar) of (a) Locke solution via lingual and thyroid catheters; (b) SP 25 µg/min via the lingual, Locke solution via the thyroid catheter; (c) met-ENK 10 µg/min via the thyroid, Locke solution via the lingual; (d) met-ENK 10 µg/min via the thyroid, SP 25 µg/min via the lingual. The accompanying blood pressure records are shown on the right-hand side of the figure, the time scale being common to both sides. The catheter dead-space needed to be cleared before the peptides arrived in the carotid artery, a process which took 1-2 min after starting the infusion, except in (d) where the thyroid catheter was fully primed with met-ENK so that the action of this peptide had begun before SP reached the carotid artery.

as well as to study the effects of infusing simultaneously adenosine and met-ENK.

Figure 4.13 illustrates the affect of an infusion of adenosine compared with an infusion of met-ENK and thereafter in the same cat the effects of both substances when the infusions were carried out simultaneously. As can be seen, adenosine alone had an excitatory effect, met-ENK an inhibitory effect, and when both were infused simultaneously a summation was observed.

Four experiments were performed to investigate the influence of naloxone (400 µg i.c.) on chemoreceptor responses to adenosine and morphine. The adenosine results were pooled and are shown in Figure 4.14. No statistically significant difference was detected between the pre- and post-naloxone response ($P < 0.05$). In two of these experiments morphine (100 µg i.c.) was injected before and after naloxone (Figure 4.14). A chemodepressant effect of morphine was obtained in accordance with previous observations (see above) and this effect was converted to an excitatory one by naloxone.

The influence of theophylline (1 mg i.c.) on the responses to morphine and met-ENK was also investigated in two experiments. As can be seen from Figure 4.15 theophylline had little or no effect on the inhibitory response to met-ENK. The total area of inhibition of spontaneous chemoreceptor discharge caused by met-ENK (10 µg i.c.) was slightly greater after theophylline than before the injection of this substance (see Figure 4.15). A small change occurred with morphine. For example, morphine 10 µg i.c. decreased spontaneous chemoreceptor discharge to 64% of the pre-injection (control) discharge in the 60 s that followed the injection and a decrease of 77% was obtained after theophylline.

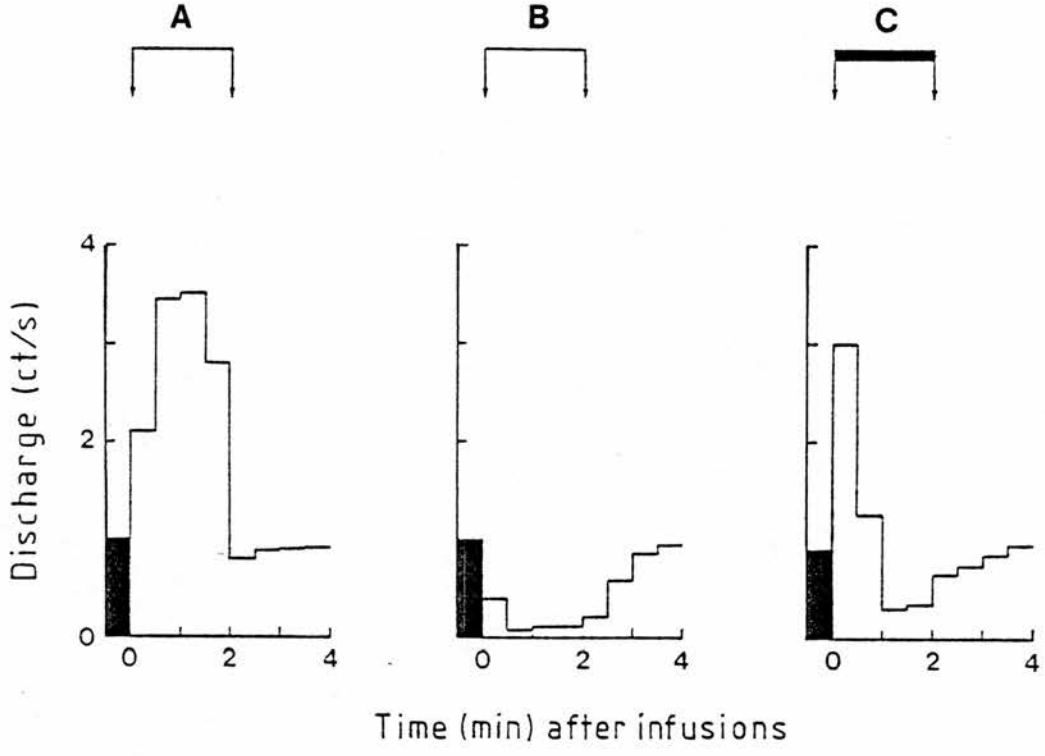


FIGURE 4.13

From left to right effects of infusing (bars between arrows) adenosine (50 µg/min) via lingual catheter (A), met-ENK (100 µg/min) via thyroid catheter (B) and concurrently adenosine (50 µg/min) via the lingual + met-ENK (100 µg/min) via thyroid catheters (C). The catheters were fully primed with adenosine or met-ENK. The horizontal bars indicate the duration of infusions, and the black rectangles the discharge averaged before starting the infusions.

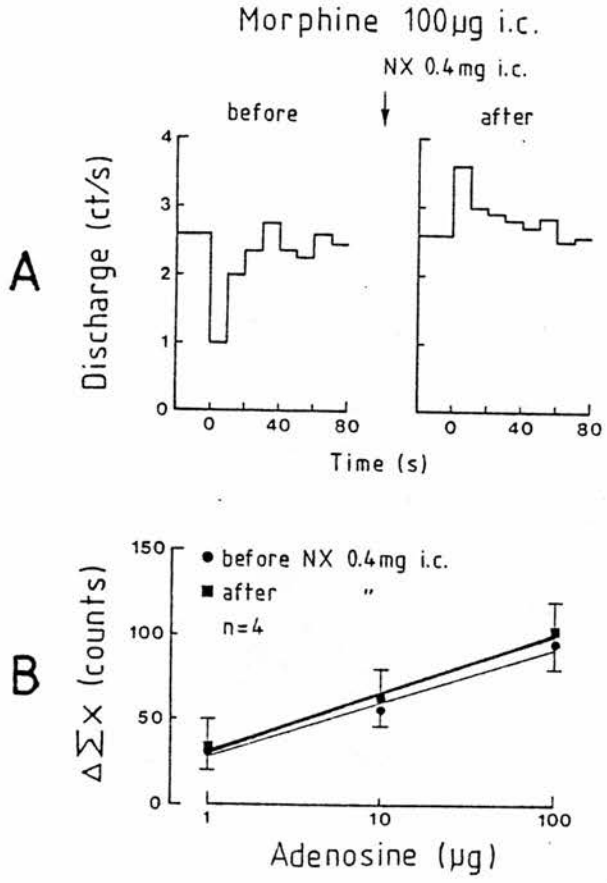


FIGURE 4.14

A) Effects of morphine (100 µg i.c.) on chemoreceptor discharge before and after naloxone (NX 0.4 mg i.c.) in one experiment. Discharge was averaged over 10 s periods following the injection. B) Dose-response data for adenosine obtained before (●) and after (■) administration of NX (0.4 mg i.c.) in four experiments. Doses (µg i.c.) are plotted on a $\log_{10}$ scale and chemoreceptor responses expressed as the mean $\Delta\Sigma x$ for the four experiments; vertical lines show s.e. mean. $\Delta\Sigma x$ was calculated as the response during the 30 s following the injections. Lines were fitted to the data by the method of least squares. Averaged values (ct/s) for the pre-injection (15 s) periods ± s.e. mean were as follows: adenosine 1 µg 4.5 ± 2.1 before NX, 5.7 ± 2.6 after NX; adenosine 10 µg 4.2 ± 1.4 before, 6.7 ± 1.5 after; adenosine 100 µg 6.5 ± 1.3 before, 5.0 ± 1.9 after.

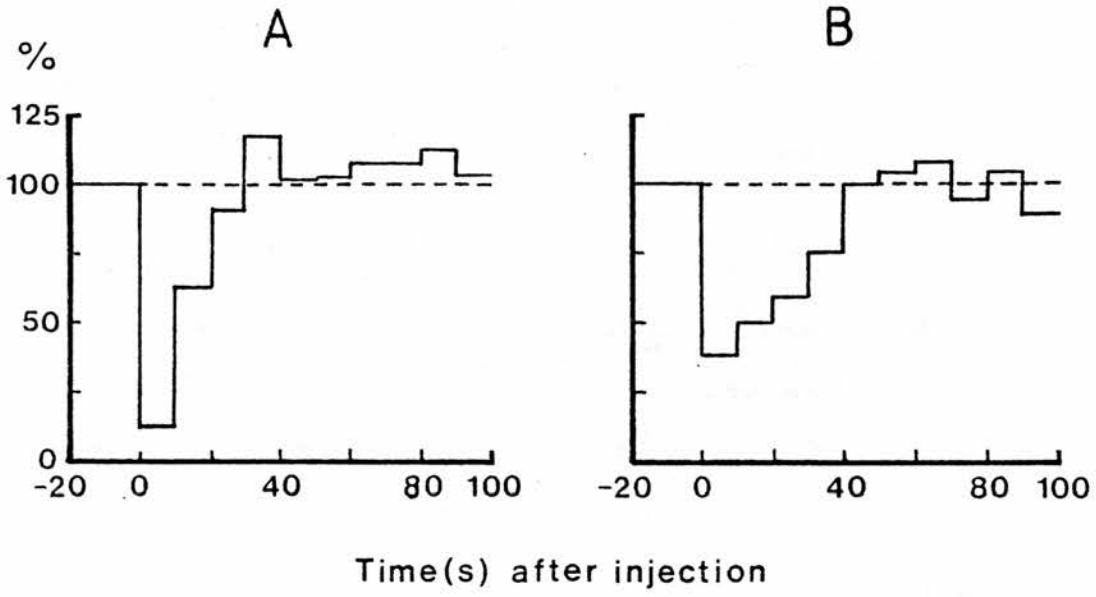


FIGURE 4.15

Effect of met-ENK 10 µg i.c. on spontaneous chemoreceptor discharge before (A) and after (B) theophylline, 1 mg i.c. Discharge was averaged over 10 s periods following the injections and expressed as a percentage of the averaged discharge in the 20 s pre-injection control period. Data from one experiment. Average values (ct/s) for the control (100%) periods are before theophylline 0.8 ct/s and after 2.3 ct/s.

Vasoactive intestinal polypeptide

The effects of VIP were studied in two cats.

Injections of VIP

Figure 4.16 illustrates the effect of injecting VIP (3.2 μg i.c.) on spontaneous chemoreceptor discharge and results from different injections are summarized in Figure 4.17. Low doses caused a decrease in discharge whereas higher doses increased it. The effects were fairly long-lasting, as can be seen from the discharge recorded in the second min following the injection (see Figure 4.17). Higher doses caused a fall in B.P. followed by a rise (Figure 4.1).

Infusions of VIP

Infusion of VIP at a rate of 0.5 μg/min i.c. for 5 to 10 min caused a sustained increase in discharge and a fall in B.P. (see Figure 4.18). The increase in chemoreceptor discharge was associated with a slight fall in B.P. The control B.P. was rather low, and to preclude the possibility that the increase in chemoreceptor discharge was secondary to the systemic vascular effects of VIP, the infusion was repeated later in the same experiment when mean systemic B.P. was over 100 mmHg and dextran was infused to prevent the fall in B.P. Under these conditions discharge still increased, although the increase was a little less than that obtained at the lower pressure. Measurement of arterial blood gases and pH showed that the increase in chemoreceptor discharge was not secondary to changes in arterial blood gas tensions. Values for pH, PaO_2 and $PaCO_2$ were respectively 7.31, 107 Torr and 34 Torr during the infusion compared with pre-infusion sample values of pH 7.28, PaO_2 105 Torr and $PaCO_2$ 36 Torr.

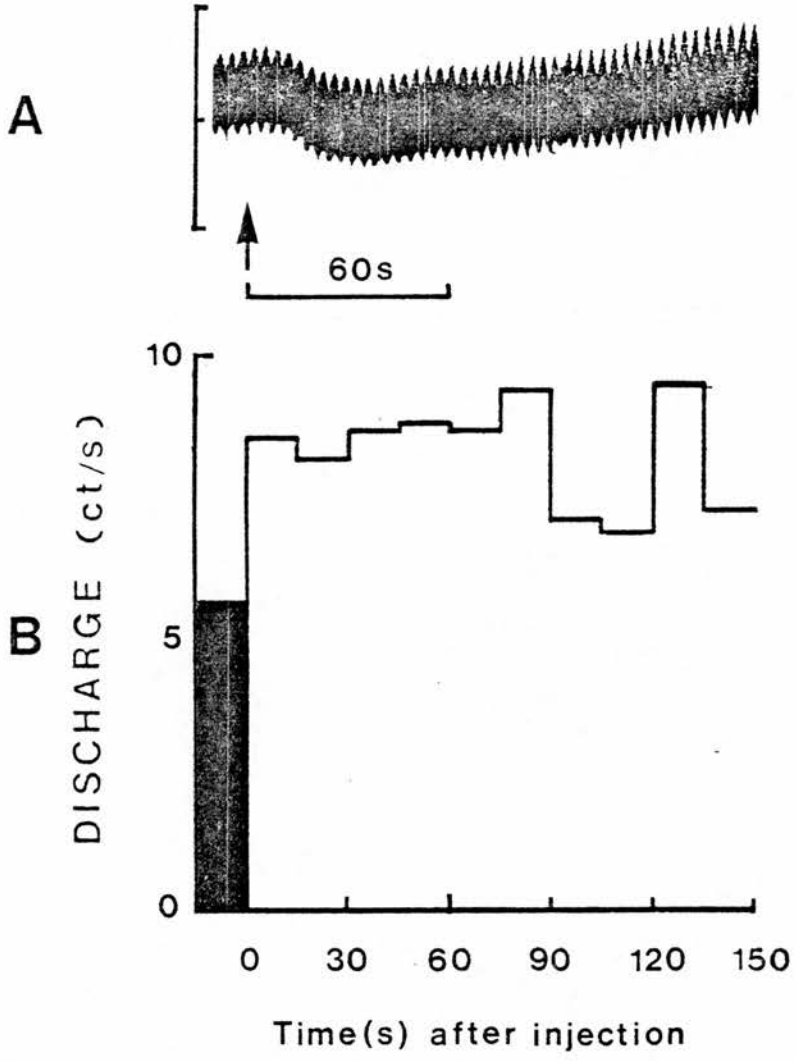


FIGURE 4.16

Effects of injecting VIP (3.2 µg i.c.) on A) systemic arterial blood pressure and B) spontaneous chemoreceptor discharge (ct/s). Discharge averaged over 15 s periods following the injection.

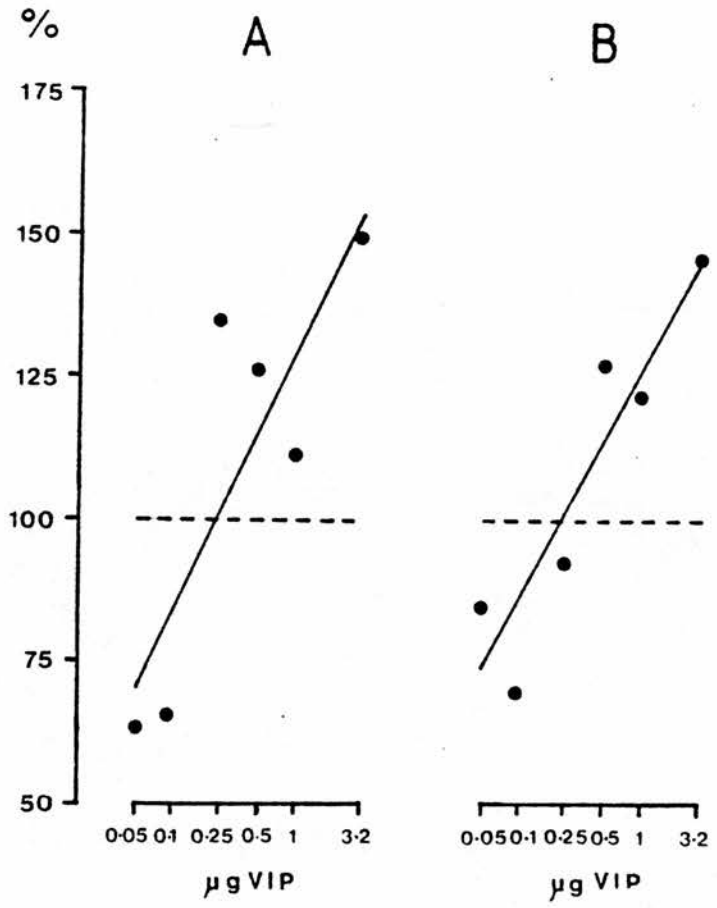


FIGURE 4.17

Effect of VIP on spontaneous chemoreceptor discharge averaged over the first (A) or second (B) min following VIP injection (100% = 5.6 ct/s). Recordings from 3 to 4 chemoreceptor units. For further details see legend of Figure 4.5.

Evoked responses during infusion of VIP

Responses to ACh, CO_2 and NaCN were determined before, during and after infusions of VIP (0.5 µg/min). Results obtained from two experiments were pooled and it was found that responses to the stimulants were reduced by VIP (ACh 50%, NaCN 57%, CO_2 65% in relation to the control responses taken as 100%), whereas the inhibitory effect of dopamine was only slightly reduced to 90% of the control during the infusion, although there was a further reduction to 75%, after infusion. Responses to ACh and CO_2 recovered within 15 min, whereas the NaCN and dopamine responses did not.

In one experiment it was investigated the influence of a VIP infusion (0.5 µg/min) on the inhibition evoked by met-ENK (10 µg i.c.). Before the VIP infusion chemoreceptor discharge averaged over the immediate 60 s post-injection period was reduced to 27% of the pre-injection control frequency, and over the second min it was reduced to 89% of control. Corresponding values during VIP infusion were 46% in the first min and 100% in the second, meaning that the inhibition caused by met-ENK was reduced during the VIP infusion.

Cholecystokinin octapeptide

The effects of CCK-8 on chemoreceptor activity were examined in two experiments.

Injections of CCK-8

Injections of CCK-8 (0.1 - 100 µg i.c.) caused a somewhat variable short-lasting biphasic effect on discharge, generally an initial decrease followed by an increase (see Figure 4.19). Higher doses caused a rise in B.P. The quantitative evidence shown in

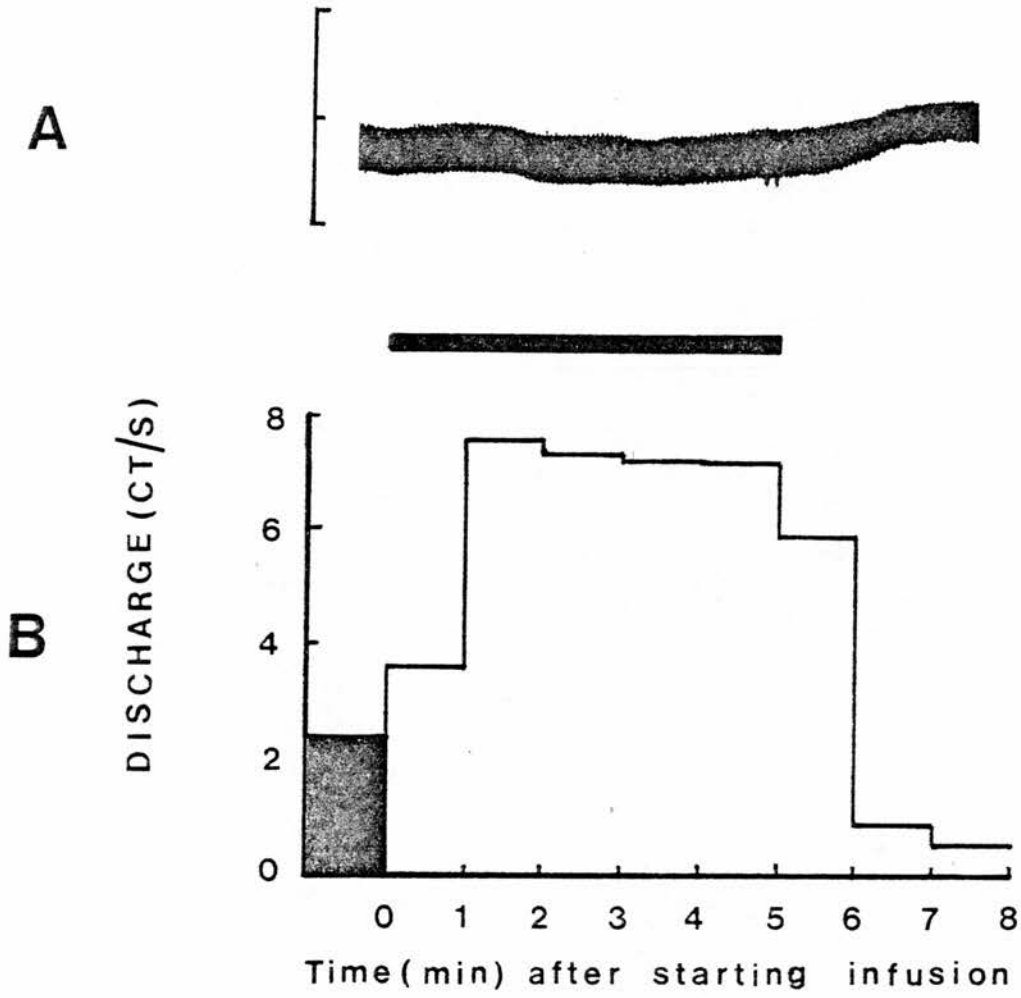


FIGURE 4.18

Effects on spontaneous chemoreceptor discharge of infusing during the period represented by the horizontal bar, VIP 0.5 µg/min i.c. Discharge was averaged over 60 s intervals, commencing 60 s before starting infusion, and plotted on the same time scale as the B.P. trace. The catheter was primed with polypeptide solution (i.e. there was no dead-space to be cleared after starting an infusion).

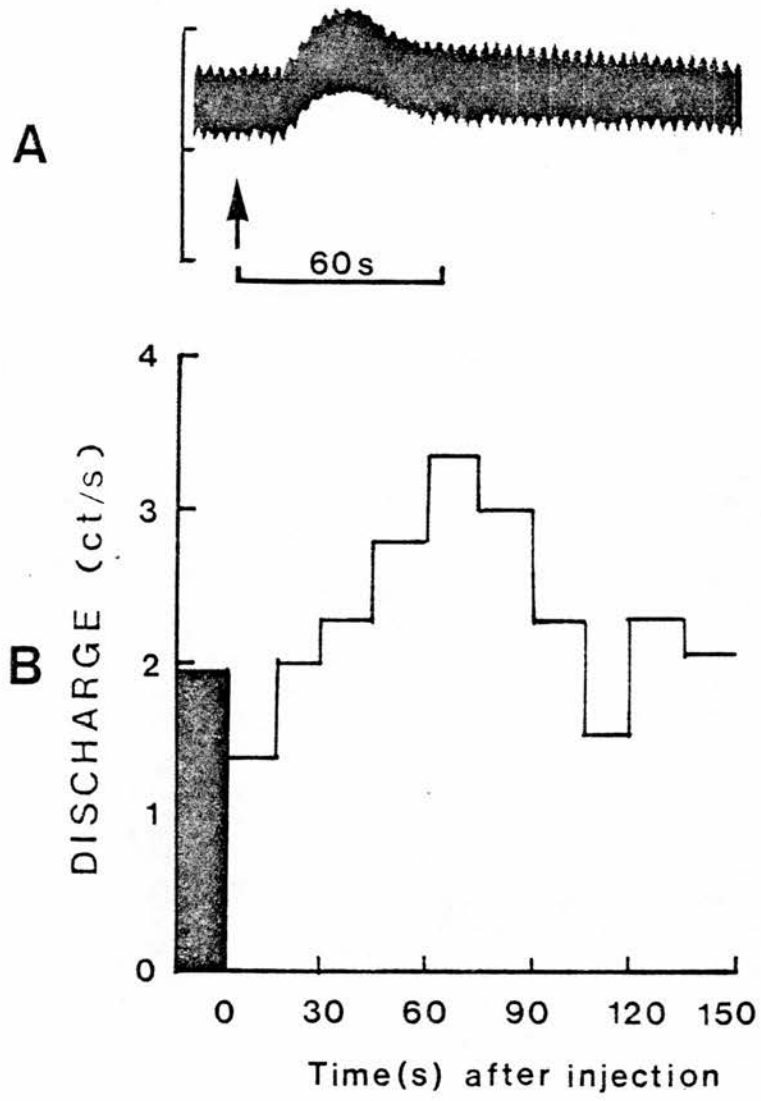


FIGURE 4.19

Effect of injecting CCK-8 (100 µg i.c.) on A) systemic arterial blood pressure and B) spontaneous chemoreceptor discharge (ct/s). Discharge averaged over 15 s periods following the injection.

Figure 4.20 A and B confirms that responses were somewhat variable and short-lived.

Infusions of CCK-8

In one experiment CCK-8 was infused at a rate of 1 µg/min, in another the rate was 10 µg/min (Figure 4.21). There was little effect on spontaneous discharge with either dose; but a marked and sustained increase in B.P. was detected after the first min post-infusion.

Evoked responses during infusion of CCK-8

Responses to ACh, NaCN and dopamine were reduced during infusion of CCK-8 at a rate of 1 µg/min. The response to CO_2 was unaffected (Figure 4.22). Although the ACh effect returned to pre-infusion levels within 15 min of finishing the infusion, responses to NaCN and dopamine remained depressed. In a second experiment during which CCK-8 was infused at 10 µg/min, responses were somewhat similar, except that a sustained potentiation of the dopamine-induced chemoinhibition was observed and there was a decreased response to CO_2 in the post-infusion period, although not during the infusion (Figure 4.22).

DISCUSSION

In this section it is shown that the polypeptides investigated can modify carotid chemoreceptor activity in the cat. Spontaneous chemoreceptor discharge was decreased by intracarotid injections of met-ENK, leu-ENK and β-endorphin and by low doses of VIP, whereas it was increased by CCK-8 and higher doses of VIP.

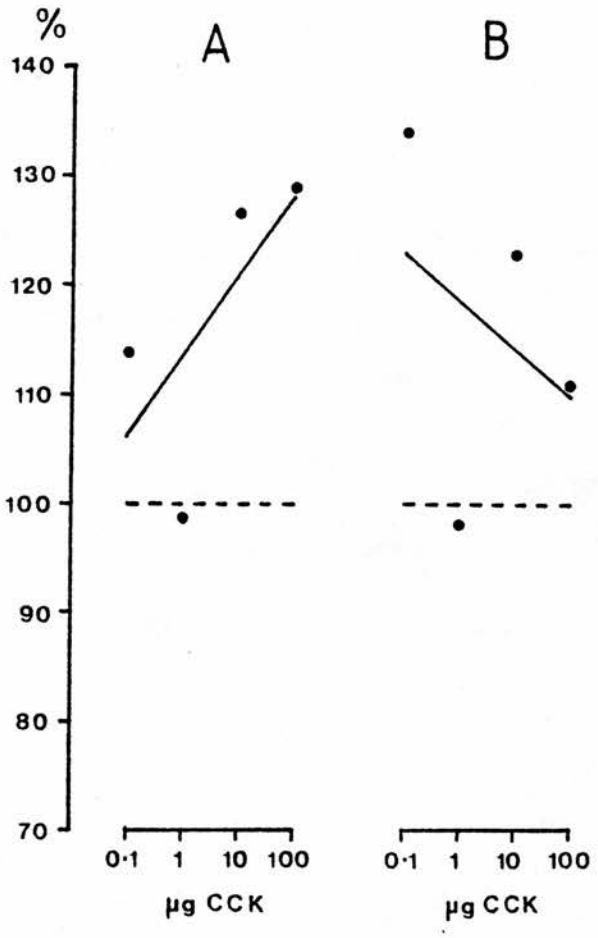


FIGURE 4.20

Effects of injecting CCK-8 on spontaneous chemoreceptor discharge in two experiments. Data were pooled and discharge was averaged over the first (A) or second (B) min following CCK-8 injection (100% = 1.9 ct/s). Figure details as for Figure 4.5.

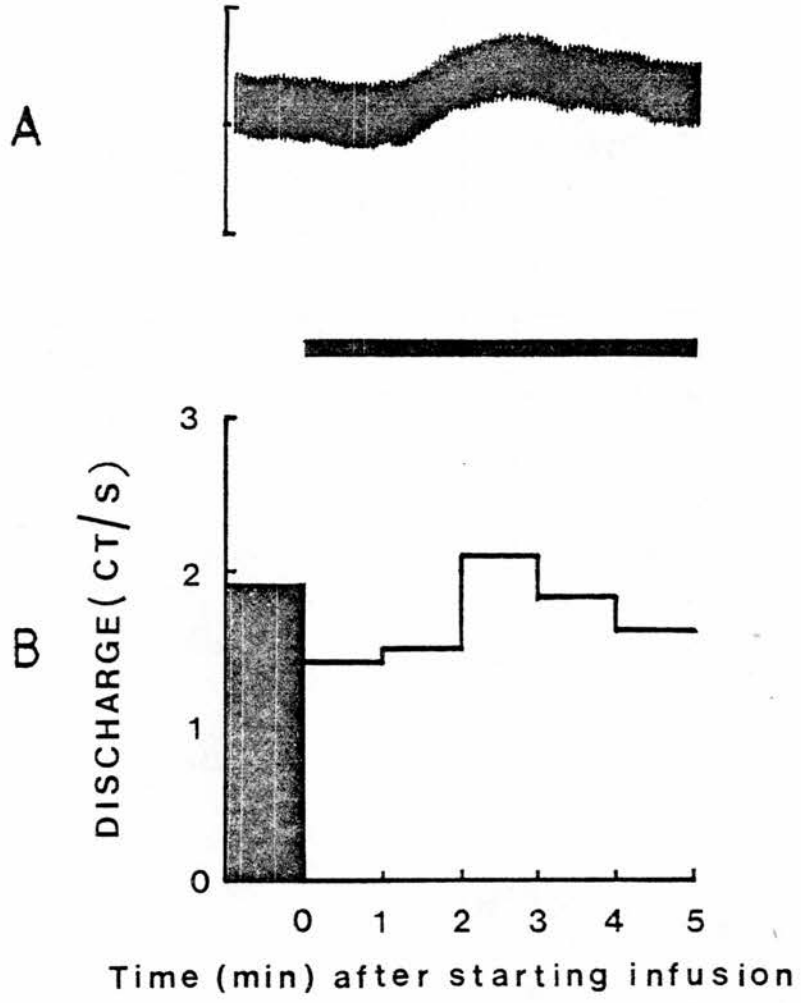


FIGURE 4.21

Effect on spontaneous chemoreceptor discharge of infusing CCK-8 10 µg/min i.c. during the period represented by the horizontal bar. Discharge (ct/s) was averaged over 60 s before starting infusion, and plotted on the same time scale as the B.P. trace. The catheter was primed with polypeptide solution (i.e. there was no dead-space to be cleared after starting an infusion).

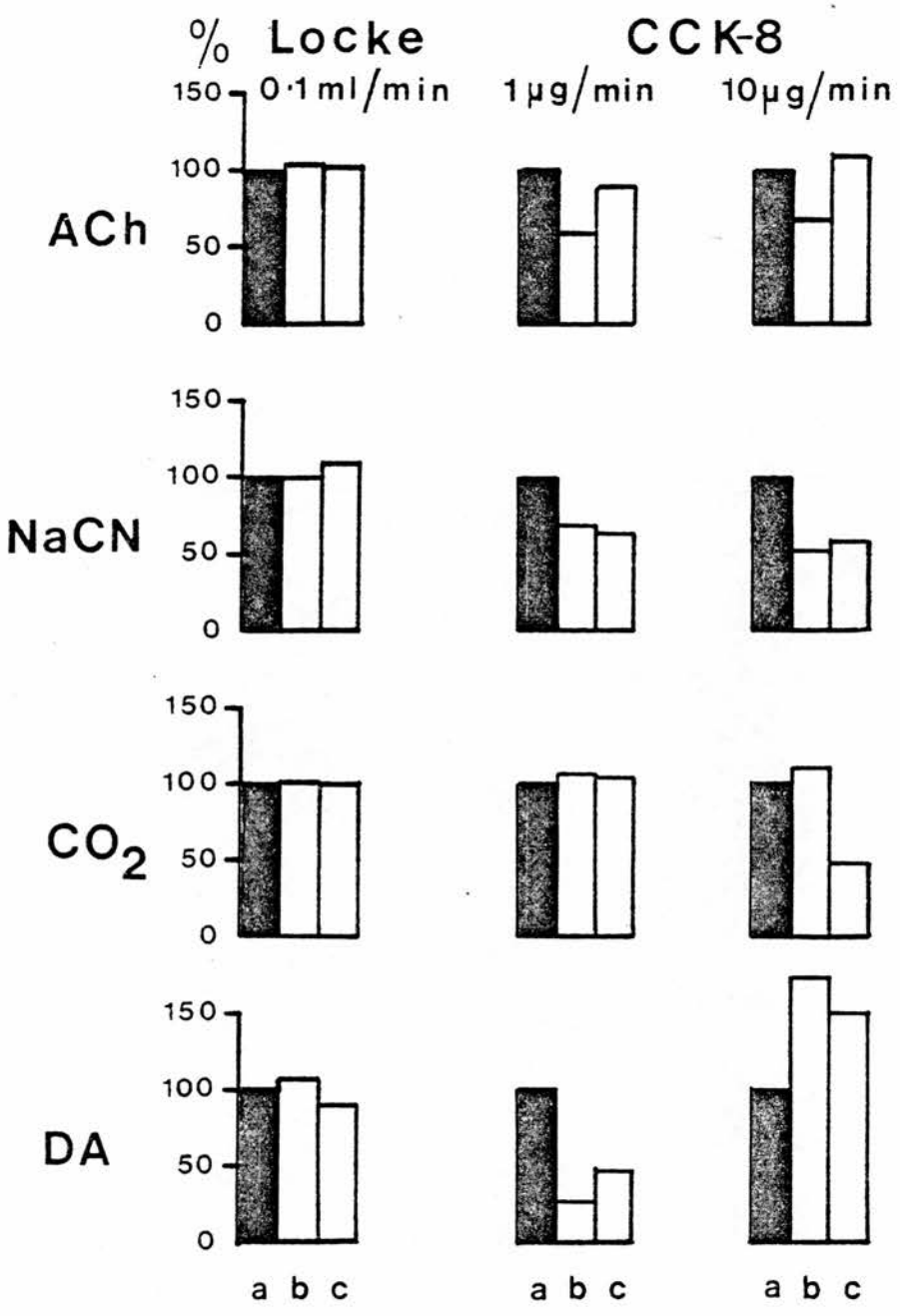


FIGURE 4.22

Responses ($\Delta\Sigma x$) to ACh (50 µg i.c.), NaCN (2.5 µg i.c.), CO_2 (0.3 ml CO_2-equilibrated Locke solution) and dopamine (DA, 5 µg i.c.) were obtained (a) before a 10 min infusion of CCK-8 into the second carotid catheter, (b) during the infusion and (c) 5 - 15 min after finishing the infusion and are shown as percentages (pre-infusion response = 100%). Data from a single experiment. For Locke solution the data shown are averages from two experiments.

Both met-, and leu-ENK inhibited spontaneous chemoreceptor discharge by acting on naloxone-sensitive receptors in the carotid body. β-endorphin and morphine also acted at these receptors, but they were less potent than the enkephalins and caused a more variable inhibition which tended to be biphasic.

Injection of the specific opiate antagonist naloxone (see Sawynok, Pinsky and Labella, 1979 for a review) slightly increased chemoreceptor discharge, an effect which does not appear to be due to reversal of residual enkephalins, β-endorphin or morphine chemodepression because it was also observed when none of the substances had been administered. This may mean that there is some tonic inhibition of chemoreceptor discharge by an opioid; alternatively, the effect could be secondary to changes (e.g. in B.P.) induced by naloxone acting elsewhere. The dose of naloxone (200 μg i.c.) is adequate for reversing the fall in B.P. caused by morphine in cats (Feldberg and Wei, 1977; McQueen, personal communication) and for antagonizing the action of enkephalins in the cat substantia gelatinosa (Duggan, Hall and Headley, 1977). In the present experiments naloxone reduced the chemoreceptor inhibition caused by the enkephalins and, to a greater extent, that caused by β-endorphin and morphine. It is known that inhibition of neuronal firing caused by morphine is more readily antagonized by naloxone than is that caused by opioid peptides (North, 1979). Increasing the dose of naloxone to 0.8 mg i.c. produced a greater decrease of the enkephalin-induced inhibition of chemoreceptor discharge, and virtually abolished the morphine effect. Higher doses of naloxone could have been studied, but they might have exerted non-specific actions, or reversed the anaesthetic (Fürst, Foldes and Knoll, 1977; Arndt and Freye, 1979; Sawynok, Pinsky and

Labella, 1979). It seems reasonable to conclude that chemoreceptor inhibition results from actions of opioid peptides, β-endorphin and morphine on a naloxone-sensitive receptor in the carotid body, whereas the slight chemoexcitation seen after naloxone does not. The latter effect may result from actions at opiate receptors which are insensitive to naloxone in the doses used, or to direct or indirect actions on non-opiate receptors.

The greater potency of naloxone as an inhibitor of morphine and β-endorphin in comparison with its effects against the enkephalins may reflect differences in the potencies of morphine and β-endorphin compared with the enkephalins and this may be due to different populations of opiate receptors. According to Lord, Waterfield, Hughes and Kosterlitz (1977) it is possible to distinguish at least two categories of opiate receptors: μ receptors which are characterized by their high affinity for morphine and related alkaloids as well as for the antagonist naloxone, whereas δ receptors preferentially recognize the enkephalins and are relatively resistant to naloxone. Confirmation of this idea comes from Fields, Emson, Leigh, Gilbert and Iversen (1980) who have shown that in the primary afferent tissue (dorsal root) and dorsal horn morphine has a higher affinity for μ-sites whereas met-, and leu-ENK have greater affinity for δ-sites. They also suggested that separate pre- and post-synaptic populations of opiate receptors exist, with a relatively greater proportion of δ-type receptors on post-synaptic elements of the dorsal horn. Interpreting the present results in the light of these findings one might suggest that in the carotid body μ and δ receptors appear to be present and δ receptors could be responsible for the greater part of the inhibition observed, and that this might result from an action on the post-synaptic component of

the sensory synapse. The μ receptors at which morphine and naloxone might act could be associated with pre-synaptic elements in the carotid body. Taking the speculation further one might suggest that the effect of morphine arises from a modification (? decrease) of transmitter release from the pre-synaptic component (e.g. dopamine, ACh) by depressing calcium uptake (see e.g. Guerrero-Munoz, Cerreta, Guerrero and Way, 1979), and the effects of the enkephalins may result from a hyperpolarization of sensory nerve endings.

Low doses of morphine tended to increase spontaneous chemoreceptor discharge, an effect which was potentiated and also obtained with higher doses of morphine, as well as low doses of the enkephalins, after naloxone. Landgren, Liljestrand and Zotterman (1952) found that an intracarotid injection of 3 mg morphine hydrochloride in cats caused a moderate increase in small action potentials (probably chemoreceptors) recorded from the sinus nerve, and Eyzaguirre and Zapata (1968a) showed that morphine caused a transient increase in discharge recorded from the *in vitro* carotid body preparation. Whether the excitation seen in the present study resulted from an action on naloxone-insensitive opiate receptors, or to direct or indirect actions on non-opiate receptors, e.g. morphine/opioid peptides, might influence substances in the carotid body, as occurs in other tissues [e.g. ACh (Paton, 1957), noradrenaline (Szerb, 1961; Snyder and Childers, 1979), 5-HT, or dopamine (Loh, Brase, Sampath-Khanna, Mar, Way and Li, 1976)].

The polypeptides (the enkephalins, β-endorphin, VIP and CCK-8) influenced arterial blood pressure and this confirmed that, in the doses studied, they were biologically active. As stated above in relation to adenosine the possibility that the effects of polypeptides on chemoreceptor

activity were secondary to vascular changes either within the carotid body, or systemically, needs to be considered. The rapid onset of the peptide effects (within 1 - 2 s of injection), the fact that effects on chemoreceptor discharge occurred before changes in blood pressure were seen and persisted when such changes were prevented makes it unlikely that vascular effects were responsible for the greater part of the responses observed (see also Section VI, General Discussion and Conclusions).

According to several authors (Hornbein, Griffo and Roos, 1961; Biscoe, Purves and Sampson, 1970; Acker, Keller, Lübbers, Bingmann, Schulze and Caspers, 1973; McQueen, 1977) the carotid body chemoreceptors are relatively unaffected by sustained changes in arterial blood pressure or in total carotid body flow within the physiological range (60 - 160 mmHg). Moreover, Acker and Lübbers (1977) could not find any consistent relationship between local blood flow and PaO_2.

Summarizing this point, one might say that it is unlikely that vascular effects are responsible for the greater part of the chemoreceptor effects of these substances, but it would be interesting to investigate their effects on the *in vitro* carotid body preparation (Eyzaguirre and Lewin, 1961) which would eliminate the vascular complications.

Although met-ENK and morphine were able to inhibit spontaneous chemoreceptor discharge, they only slightly reduced excitatory responses evoked by ACh and NaCN, and potentiated dopamine-induced inhibition. These were long-term effects since the responses were not studied until 5 - 20 min after injections of met-ENK or morphine. When responses were studied during infusions of met-ENK and β-endorphin the excitatory effect of NaCN was reduced whereas the responses to ACh and CO_2 were potentiated by met-ENK but reduced by β-endorphin.

Responses evoked by ACh, NaCN and CO_2 were also studied during infusions of VIP and CCK-8, when the levels of these substances in the carotid body were assumed to be relatively steady, showed that the excitatory action of NaCN and ACh was reduced, and that caused by CO_2 was reduced by VIP, but unaffected by CCK-8.

Dopamine-induced chemoinhibition was potentiated by β-endorphin, largely unaltered by VIP, reduced by low concentrations of CCK-8 and potentiated by higher concentrations.

These single-dose studies are difficult to interpret (see Section II, Methods and Materials), particularly since polypeptides may alter blood flow through the carotid body, but the point is that the peptides so far studied influence evoked responses.

The chemoinhibitory effect of low doses of the enkephalins was very similar to that obtained with dopamine (5 μg i.c.), although it should be noted that the enkephalins are 10 - 100 times more potent on a molar basis. However, despite the similarities, it is unlikely that the enkephalins act directly, or, by releasing dopamine within the carotid body, indirectly at a dopamine receptor, because α-flupenthixol blocks the inhibitory action of exogenous dopamine (Docherty and McQueen, 1978) without affecting the inhibitory response to met-ENK (McQueen, 1981).

SP is present in the cat carotid body glomus type I cells and carotid sinus nerve (Cuello and McQueen, 1980) and causes an increase in chemoreceptor discharge when injected i.c. (McQueen, 1980). It may be that the enkephalins are acting to inhibit the release of SP within the carotid body in the same way as has been shown to occur in the central nervous system (Jessel and Iversen, 1977). During the present study it was investigated whether any interaction occurred in the carotid body between SP and met-ENK. SP has been suggested to

be a transmitter at the central nerve terminals of chemoreceptor afferent fibres of cats (Gillis, Helke, Hamilton, Norman and Jacobowitz, 1980) and rats (Helke, O'Donohue and Jacobowitz, 1980; Jacobowitz and Helke, 1980). It is also known that met-ENK (also present in the cat carotid body, see Section I, General Introduction) can inhibit the release of SP from cultured sensory neurones (Mudge, Leeman and Fishbach, 1979) and probably in the central nervous system (Jessel and Iversen, 1977). The present results showed that SP was unable to overcome the chemoinhibitory effect of met-ENK, something it might have been expected to do if release of endogenous SP is crucial for chemoexcitation, and can be prevented by met-ENK. However, much may depend on the type of fibre being recorded and the background level of activity; SP is particularly associated with C fibres (Hökfelt, Johansson, Kellerth, Ljungdahl, Nilsson, Nygårds and Pernow, 1977) and it could be that A fibres were being recorded which are, perhaps, affected by met-ENK, but not SP. In the absence of conduction velocity experiments this must remain speculation, but also is unknown whether following injection of SP the concentration in the carotid body was physiological. As far as the other polypeptides were concerned, the inhibition evoked by met-ENK was largely unaffected by β-endorphin but reduced by VIP.

In contrast to what has been suggested for the smooth muscle (Sawynok and Jhamandas, 1976) and the brain (Stone and Perkins, 1979) adenosine does not appear to be the mediator of carotid chemo-inhibitory action of opiates because: (1) adenosine did not mimick the action of morphine or the enkephalins. These substances cause a rapid and potent inhibitory action on chemoreceptor activity whereas adenosine causes a rapid increase on chemoreceptor discharge;

(2) naloxone antagonized the inhibitory action of opiates but did not cause substantial modifications in the excitatory action of adenosine; (3) theophylline did not cause substantial changes in the inhibitory action of opiates and potentiated the adenosine chemoexcitatory action (see also Section III).

The effects of polypeptides such as β-endorphin, VIP and CCK-8 on chemoreceptor discharge may not seem very impressive when compared with those of ACh, NaCN, CO_2 or dopamine, but it has to be remembered that the polypeptides have relatively high molecular mass (see Section I, General Introduction). For example, the excitatory response to ACh can be elicited by 180 nmoles (50 μg) (see e.g. McQueen, 1977), that to VIP involved only 1 nmole (3.2 μg). Furthermore, although the peptide effects were generally less intense they tended to be longer lasting than those associated with classical neurotransmitters. The prolonged action may be related to various factors, such as molecular size, tissue penetration, dissociation from receptors, activation of 'second messengers', or to peptide synthesis and inactivation.

Evidence from other parts of the nervous system suggests that some neuropeptides may function as neurotransmitters (see e.g. Hökfelt, Johansson, Ljungdahl, Lundberg and Schultzberg, 1980), but the question of what physiological role the neuropeptides play in the nervous system is still very much a matter for debate (see Bishop and Polak, 1978; Guillemin, 1978; Hökfelt, Johansson, Ljungdahl, Lundberg and Schultzberg, 1980; Snyder, 1980). At the carotid body, various exogenous neuropeptides alter chemoreceptor activity and several of these are known to be present in the structure. However, on its own this information is not enough to reach a conclusion regarding

the role of the peptides in the carotid body. They may function as neurotransmitters, neuromodulators, cotransmitters, neurohormones, trophic factors, or as agents modifying the vasculature, they might be influenced by each other, by carotid body amines, by circulating hormones (e.g. ACTH, β-endorphin) or by other substances.

In conclusion, the present results provide pharmacological evidence for the presence of opiate receptors in the cat carotid body. What type of receptors they are (e.g. Lord, Waterfield, Hughes and Kosterlitz, 1977), in which structural elements are located, what the endogenous ligand is and where it originates, as well as the circumstances under which it is released, all need to be investigated before one can determine whether the enkephalins or other opioid peptides have a role as neurotransmitters or neuromodulators (Kosterlitz and Hughes, 1975; Snyder and Childers, 1979) in the cat carotid body chemoreceptors. In relation to the other polypeptides studied, e.g. β-endorphin, VIP and CCK-8, the absence of drugs which can selectively affect their actions (e.g. by destruction of nerve cells, blockade of receptors, inhibition of enzymatic destruction, interference with biosynthesis) makes the proposals of their roles in chemosensory transmission still more difficult, but their long-lasting effects and increased delay to onset a chemoreceptor response, seem to suggest that they act as neurohormones rather than as neurotransmitters in the carotid body.

SECTION V

The Effects of Ouabain

INTRODUCTION

The carotid body chemoreceptors are highly sensitive to metabolic inhibitors (see e.g. Heymans, 1955; Comroe, 1964). In spite of that, little is known about the actions on arterial chemoreceptors of ouabain, a drug which has many similarities with metabolic inhibitors. In the paper by McLain (1970), it is reported that ouabain *in vivo* increases the total neural traffic recorded from the carotid sinus nerve; this includes chemo and baroreceptor activity. Other observations have been reported by Joels and Neil (1968) who found, using vascularly isolated cat carotid bodies, that ouabain decreases chemoreceptor activity evoked by nitrogenated solutions, and by Eyzaguirre, Baron and Gallego (1977) who described that *in vitro* ouabain depolarizes some carotid body type I cells.

The opportunity was taken to investigate further the effects of ouabain on cat carotid body chemoreceptors and whenever possible those effects have been quantified.

RESULTS

Injections of ouabain

The effects on spontaneous chemoreceptor discharge of different doses of ouabain (0.625 - 200 µg i.c.) are summarized in Figure 5.1. In three cats successive (cumulative) injections of ouabain were made every 15 - 25 min: in two of the cats only the lowest and the highest doses (50 - 200 µg) were used. Ouabain caused a dose-related increase in discharge (Figure 5.1), which was followed, the higher doses, by a gradual decline in discharge and the chemoreceptors became unresponsive to further injections of ouabain.

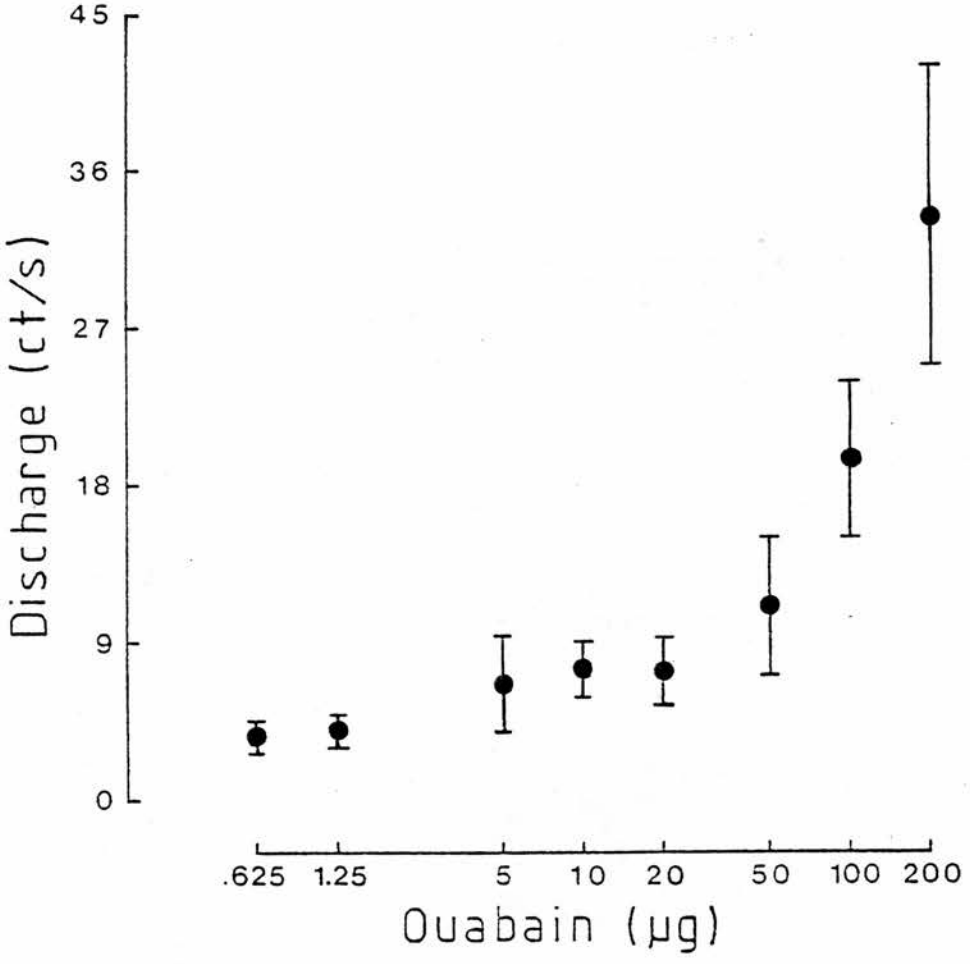


FIGURE 5.1

Effects of intracarotid injections of ouabain on spontaneous chemoreceptor discharge (ct/s). Each point is the average ± s.e. mean from two to three experiments. Averaged values (ct/s) ± s.e. mean for the pre-injection (control) discharge are as follows: 0.625 µg 2.4 ± 0.1, n=2; 1.25 µg 2.8 ± 0.9, n=3; 5 µg 5.6 ± 2.9, n=3; 10 µg 2.2 ± 0.5, n=3; 20 µg 4.0 ± 0.7, n=3; 50 µg 6.3 ± 1.2, n=2; 100 µg 9.4 ± 4.2, n=2; 200 µg 23.5 ± 10.1, n=2.

Difficulties associated with interpreting the results from these pilot experiments in which ouabain was injected led to the use of ouabain infusions as described in the next part of this section.

Infusions of ouabain

A typical response of chemoreceptors to ouabain infusion in a cat with the ganglioglomerular (sympathetic) nerves intact is shown in neurogram form in Figure 5.2, and in graphical format in Figure 5.3. Results from another six experiments in which ganglioglomerular nerves were also kept intact were pooled and are shown in Table 5.1. Ouabain solution (30 μg/ml) was infused into the common carotid artery via the superior thyroid artery at a rate of 0.1 ml (3 μg)/min. NaCN (5 μg) was injected before starting ouabain infusion, via the superior thyroid and lingual catheters; the responses were compared (Table 5.2) in order to determine whether catheter position affected chemoreceptor discharge. No significant differences were detected in relation to $\Delta\bar{x}$, and duration of the response; the mean time to onset of response was greater by 0.7 s for the thyroid catheter, though this was not statistically significant ($P > 0.05$, paired t-test) (see Table 5.2).

Infusion of the drug vehicle (Locke solution, 0.1 ml/min), via the superior thyroid artery (control experiment) did not cause substantial changes in the frequency of spontaneous chemoreceptor discharge (Figure 5.3). Thus, marked changes in the discharge after starting ouabain infusion were attributable to ouabain; not the drug vehicle. Ouabain infusion caused the frequency of spontaneous chemoreceptor discharge to increase slightly in the first 20 min of the infusion in the experiment illustrated in Figure 5.3. Thereafter, the discharge

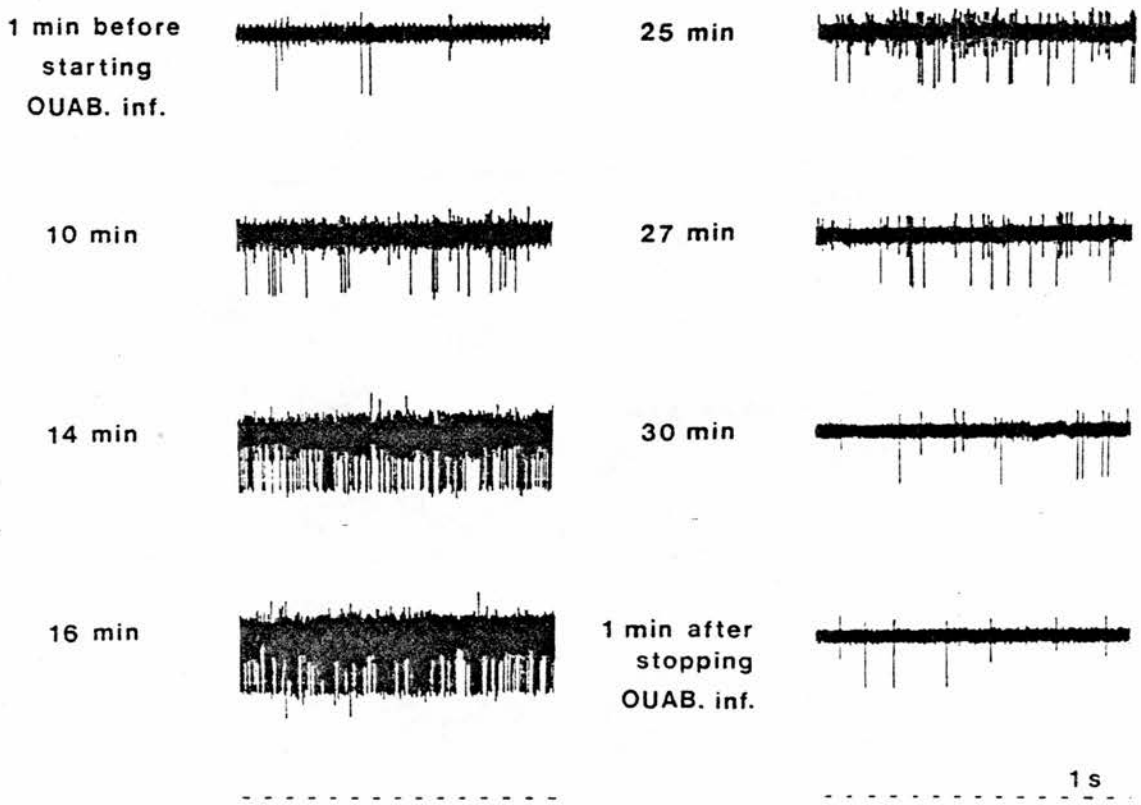


FIGURE 5.2

Effect of an infusion of ouabain (OUAB. inf.) (3 μg/min i.c.) on the frequency of spontaneous chemoreceptor discharge. Neurograms were recorded at the times indicated. The broken line at the bottom is the time calibration.

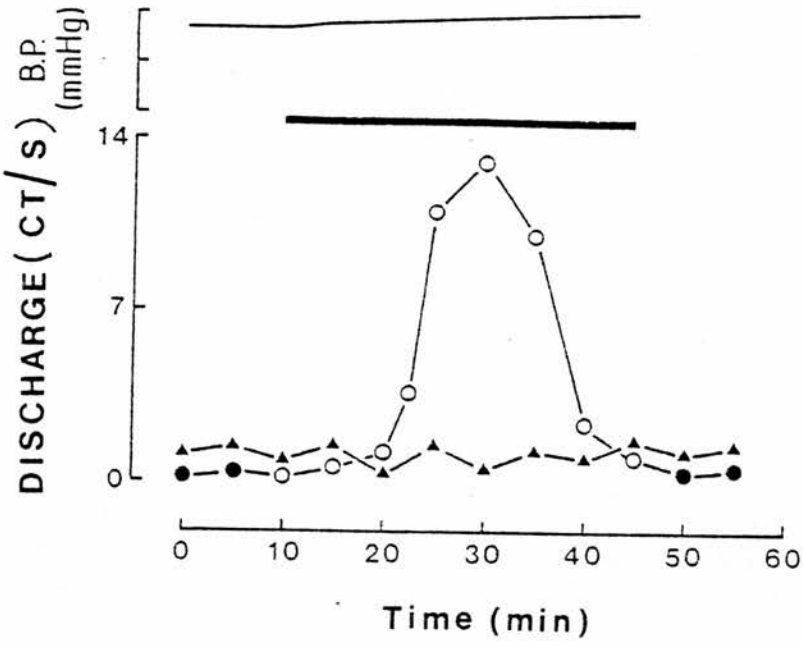


FIGURE 5.3

Effect of a ouabain infusion (3 µg/min) i.c.) on spontaneous chemoreceptor discharge in a cat with the ganglioglomerular nerves intact. The ordinates are the computed averages of the number of spikes recorded continuously during periods of 30 to 60 s. The abscissae are the times the averaging began. (▲) Locke infusion (0.1 ml/min); (●) discharge before or after ouabain infusion; (o) and during ouabain infusion. The duration of ouabain infusion is indicated by the horizontal bar. The upper part shows the mean arterial blood pressure (B.P.) recorded concurrently. B.P. calibration 0-100-200 mmHg. The mean values (3 determinations) for PaO_2, $PaCO_2$ and pH determined before and during ouabain infusion (at about the peak discharge) are as follows: before, PaO_2 94 Torr; $PaCO_2$ 31.3 Torr; pH 7.37. During PaO_2 95 Torr; $PaCO_2$ 29.5 Torr and pH 7.34.

continued to rise and 3 to 5 min later a sudden and marked increase occurred. The ouabain-induced increase in the spontaneous discharge was followed by a decline and a return to a level of the same order or slightly below that recorded before starting ouabain infusion. The discharge remained at such a level despite stopping the infusion. Chemoreceptor discharge was followed post-mortem in five animals in which death was caused by infusing ouabain. During the 10 to 40 min that followed the cardiac arrest, chemoreceptors continued to discharge though the frequency tended to decay during these periods.

Figure 5.3 also shows the mean femoral arterial blood pressure recorded simultaneously with the chemoreceptor discharge. The overall effect was an increase in B.P. which began to be apparent a few min (5 to 10 min) after starting the ouabain infusions. The averaged ± s.e. mean B.P. recorded at the time of the peak discharge for the seven experiments was 175 ± 12 mmHg, the control (obtained before starting ouabain infusion) being 142 ± 11 mmHg (see Table 5.1). The difference, 33 ± 8.3 mmHg was statistically significant ($P < 0.05$, paired t-test).

Under the present experimental conditions (extracellular recordings) no substantial modification of either the amplitude or duration of the action potentials was detected after starting ouabain infusions.

The effects of ouabain were not a consequence of changes in PaO_2, $PaCO_2$ or pH of the blood since no substantial changes in these parameters occurred during ouabain infusions compared with the pre-infusion (control) levels (see Table 5.3).

In the experiment illustrated in Figure 5.4 chemoreceptor discharge was averaged over 60 s periods; a single unit and several

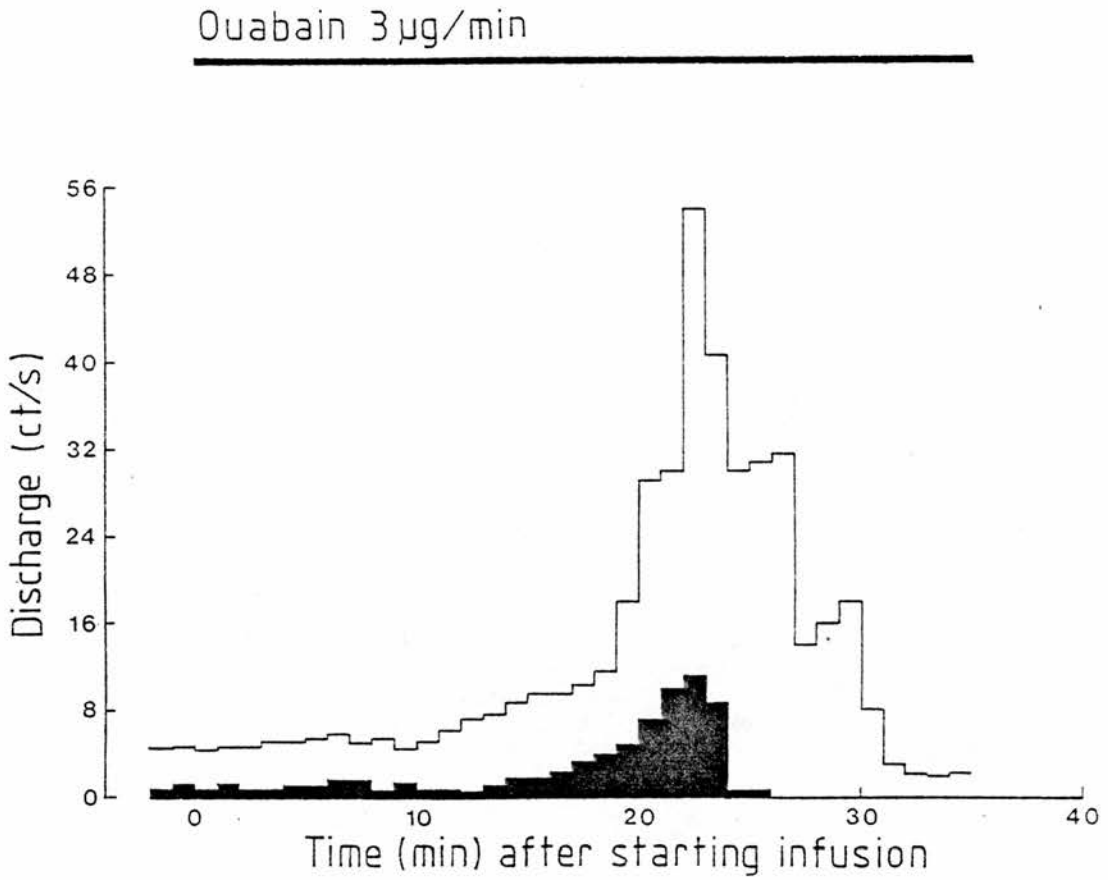


FIGURE 5.4

Effects of ouabain (3 µg/min i.c.) on spontaneous chemoreceptor discharge in a cat with the ganglioglomerular nerves intact. Chemoreceptor discharge was evaluated by counting either a single chemoreceptor unit (black) or several units (3 - 5) (upper line) from a multi-unit recording. The horizontal bar indicates the duration of infusion. Note that the pattern of the increased discharge is similar either counting a single or several units but the single unit stopped firing earlier than the other units and ceased entirely.

units were counted separately from the same multi-unit recording. As can be seen the general pattern of chemoreceptor discharge was not markedly different counting either the single or multiple units, although the single unit stopped firing earlier and it stopped discharging sooner than the other units.

Ouabain reduces carotid blood flow and increases vascular resistance (Treat, Ulano and Jacobson, 1970). According to these authors this vasoconstrictor effect is only observed during infusion of ouabain. After cessation of ouabain infusion, blood flow and resistance return toward initial control values, although ouabain is still present in the general circulation. Confirmation of a direct effect of ouabain on vascular beds has also been obtained by others (e.g. Ribeiro, 1976). In an attempt to test whether the action of ouabain on chemoreceptor activity could be related to such a mechanism, two experiments were performed in which the ouabain infusion (3 μg/min i.c.) was stopped after 10 min. The results from one of these experiments are shown in Figure 5.5. The excitatory action of ouabain was still apparent after stopping the infusion, the effect being similar to that observed in experiments in which ouabain was infused for longer periods. Figure 5.5 also shows the effect of another two infusions, each of 10 min duration, the first separated from the second by 20 min and the third from the second by 10 min. The second infusion induced a short-lasting increase in the chemoreceptor discharge and the third caused little or no effect on discharge. Following the latter infusion no chemoreceptor responses were obtained to further ouabain infusions or to substances such as NaCN (5 μg i.c.), CO_2-equilibrated Locke solution (0.3 ml i.c.), ACh (50 μg i.c.) or dopamine (5 μg i.c.) (see section below on evoked responses during uninterrupted ouabain infusions).

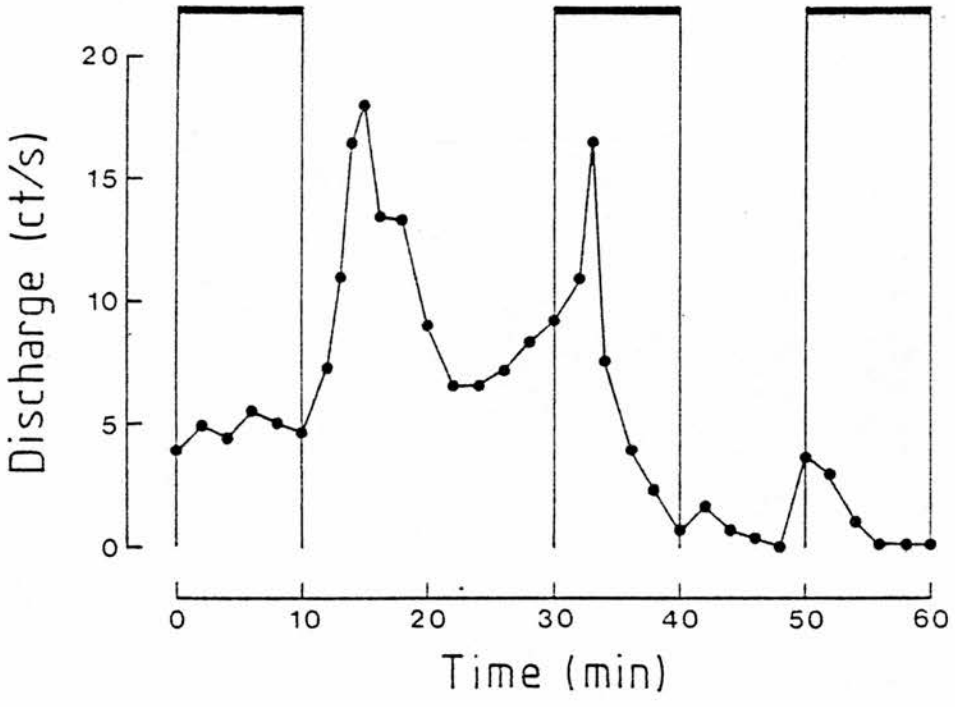


FIGURE 5.5

Effect on spontaneous chemoreceptor discharge (ct/s) of three consecutive ouabain infusions (3 µg/min i.c.) during periods of 10 min indicated by the horizontal bars, in a cat with the ganglioglomerular (sympathetic) nerves intact. Recordings from 2 to 3 chemoreceptor units. Note the increase in the discharge after stopping the first ouabain infusion, the decrease in the intensity and duration of the excitation during the second infusion and the absence of effect during the third infusion of ouabain.

TABLE 5.1: Influence of the ganglioglomerular (sympathetic) (GG) nerves on the effects of ouabain on chemoreceptor discharge

Experimental conditions		n	Control (ct/s)	Ouabain effect at the peak (ct/s)	Dose at the peak (µg/kg)	B.P. (mmHg): control	B.P. (mmHg): at the peak
Ouabain i.c.	Group 1 GG intact	7	3.1 ±1.1	17.2 ±3.6	18.7 ±1.8	142 ±11	175* ±12
	Group 2 GG cut	6	2.2 ±0.8	16.9 ±3.2	36.8† ±3.5	141 ± 9	179* ±10
Ouabain i.v. [1]	Left side GG intact	3	5.0 ±0.5	55.0 ±9.7	67.9** ±11.3	165 ±18	219* ±17
	Right side GG cut		5.5 ±0.9	36.0 ±11.4	65.6** ±16.8		

Data expressed as mean ± s.e. mean.

* P <0.05 t-test compared with control
† P <0.05 t-test compared with group 1
** P <0.05 t-test compared with i.c. administration

[1]Recordings from both carotid sinus nerves (left and right sides) were obtained simultaneously. Ouabain was infused at the same rate as i.c. (3 µg/min i.v.). Lingual arteries of both sides were cannulated.

TABLE 5.2: Comparison of the chemoreceptor responses evoked by NaCN (5 µg) injected into the common carotid artery via either the lingual or superior thyroid artery ipsilateral to the sinus nerve from which activity was recorded.

	Control	Effect of NaCN:			
	$\bar{x}$ (ct/s)	$\Delta\bar{x}$ (ct/s)	$\Delta\Sigma x$ (counts)	Duration (s)	Delay to onset (s)
Lingual	1.7 ±0.4	28.2 ±6.3	161 ±62	5.3 ±0.4	2.6 ±0.4
Thyroid	1.3 ±0.5	27.9 ±10.6	153 ±65	5.3 ±0.3	3.3 ±0.4

Data from five experiments expressed as mean ± s.e. mean.

The injections were performed at different times and the tip of the lingual catheter was about 1 cm and that of the superior thyroid catheter was about 4 cm below the carotid bifurcation (for further details see Section II, Methods and Materials).

TABLE 5.3: Effects of ouabain infusion (3 µg/min i.c.) on blood gases (PaO_2 and $PaCO_2$) and pH.

	PaO_2 (Torr)	$PaCO_2$ (Torr)	pH
Before ouabain	90.3 ±3.2	29.7 ±1.3	7.34 ±0.02
During ouabain infusion[1]	90.7 ±2.3	31.7 ±0.9	7.30 ±0.02

Data from seven experiments expressed as mean ± s.e. mean.

[1]The blood samples for these determinations were taken during the peak discharge which was usually observed within 15 to 25 min after starting ouabain infusions.

Atmospheric pressure: 724-762 mmHg.

Does the decay phase of the ouabain response result from exhaustion of chemoreceptors?

To test this possibility an experiment was designed in which Locke solution (0.1 ml/min i.c.) was infused, and the animal made hypoxic by breathing 10% O_2 : 90% N_2. As a result of this procedure chemoreceptor discharge increased markedly (Figure 5.6). The peak discharge occurred about 5 min after switching to the hypoxic gas mixture and remained elevated throughout the period of hypoxic stimulation (30 min). Thereafter hypoxia was intensified by ventilating the cat with 100% N_2 for 90 s; a further increase in the discharge was then observed (Figure 5.6). On switching to breathing O_2 (100%) for the next 60 s it was observed that chemoreceptor discharge returned to a level below that recorded before starting the hypoxic procedure. This protocol was an attempt to mimick the evolution of the discharge during ouabain infusion. After chemoreceptor discharge returned to a steady level, which occurred after 10 min of air-breathing, an infusion of ouabain (3 µg/min) was initiated. The glycoside caused an increase in discharge (Figure 5.6) with the same pattern as that described previously (see e.g. Figure 5.3).

The effect of ouabain during hypoxia

The effect of ouabain (3 µg/min i.c.) during hypoxic stimulation (15% O_2 + 85% N_2) is illustrated in Figure 5.7. An infusion of Locke solution was used as control and the hypoxic stimulation applied in its presence. Within 5 min of switching to the hypoxic gas mixture chemoreceptor discharge increased to a level where it remained more or less steady throughout the hypoxic stimulation period (30 min). Thereafter the discharge returned to the pre-hypoxia levels. Hypoxic

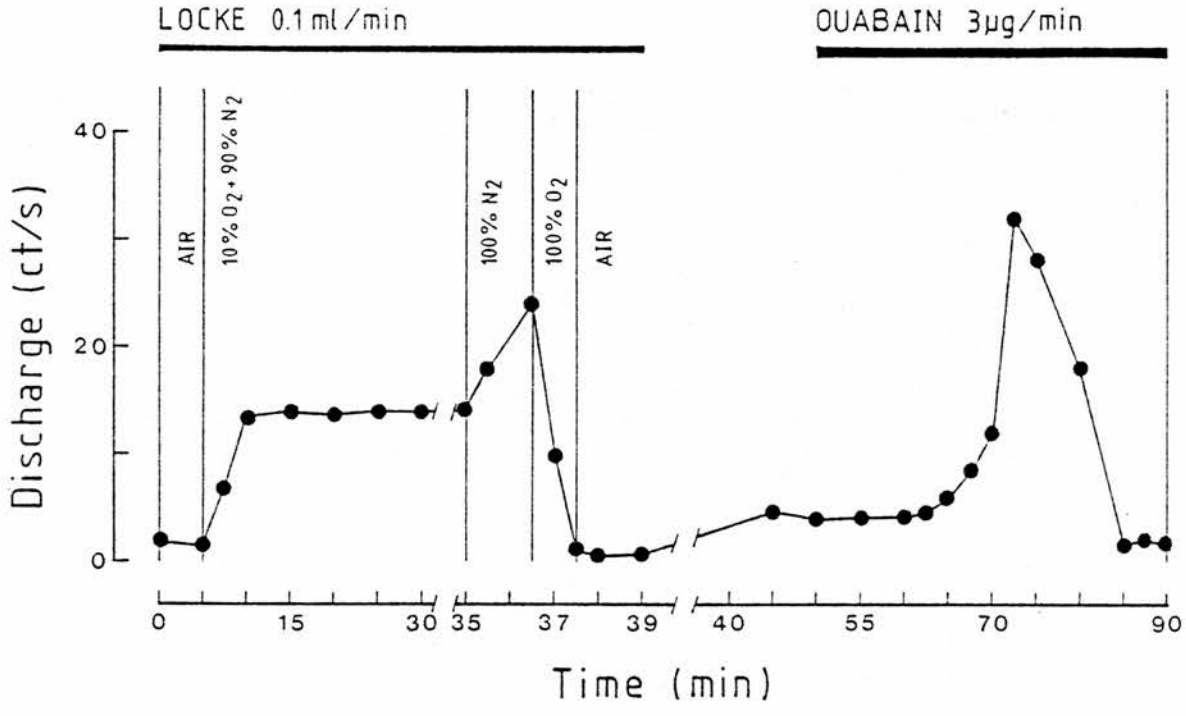


FIGURE 5.6

Effect on chemoreceptor discharge of hypoxic stimulation (breathing 10% O_2 + 90% N_2) followed by 100% N_2 and then hyperoxia (100% O_2) in the presence of a Locke solution infusion (0.1 ml/min i.c.). After discharge returned to a steady level on breathing air, ouabain (3 µg/min i.c.) was infused. The duration of the Locke solution and ouabain infusions is indicated by the horizontal bars.

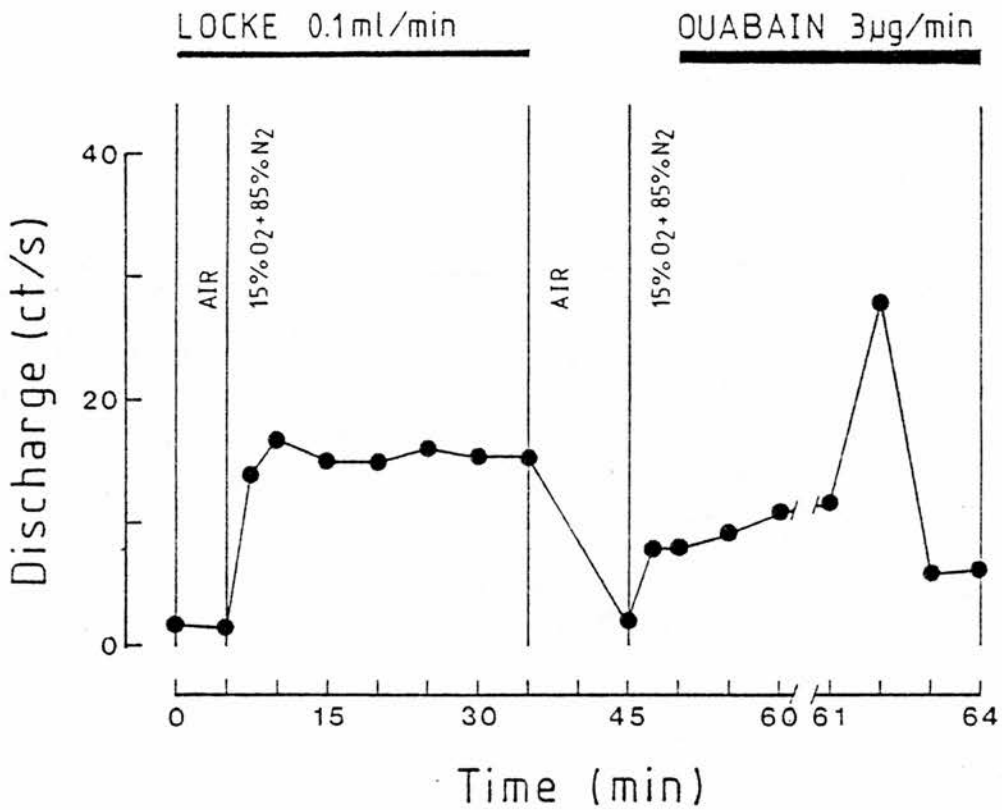


FIGURE 5.7

Effect of a ouabain infusion (3 µg/min i.c.) on chemoreceptor discharge evoked by hypoxic stimulation (15% O_2 + 85% N_2) is illustrated in the right hand side of the figure. The left hand side shows the effect of a hypoxic stimulus (15% O_2 + 85% N_2) on spontaneous chemoreceptor discharge in the presence of a Locke solution infusion (0.1 ml/min i.c.). The duration of both Locke solution and ouabain infusions is indicated by the horizontal bars. Note that the increase in chemoreceptor discharge caused by hypoxic stimulus during which ouabain was infused (right hand side of the figure) was less intense than that observed during the previous hypoxic stimulation (left hand side).

stimulus was again applied and when a steady chemoexcitatory effect was obtained an infusion of ouabain was begun. Under these conditions ouabain caused a slight increase in the discharge in the first 10 min after starting the infusion followed by a short-lasting peak (≃ 120 s) and a decay in chemoreceptor discharge to a level slightly below that recorded before starting ouabain infusion.

The influence of the ganglioglomerular (sympathetic) nerves on the effects of ouabain on chemoreceptors

Results from experiments in which the influence of the ganglioglomerular (sympathetic) nerves was studied on the chemoreceptor action of ouabain are summarized in Table 5.1. Ouabain was administered in two different ways: i.v. or i.c. close to the carotid body from which nerve activity was being recorded. For each route of administration a comparison was made between cats with intact and cut ganglioglomerular (sympathetic) nerves. It is evident that the mean dose of ouabain infused i.c. needed to increase maximally chemoreceptor discharge in cats with ganglioglomerular (sympathetic) nerves cut is significantly greater ($P < 0.05$, t-test) than in cats with the ganglioglomerular (sympathetic) nerves intact (Table 5.1). Such a difference, however, was not detected when ouabain was infused i.v. and recordings obtained from intact and denervated carotid bodies in some experiments (Table 5.1).

The carotid body chemosensory activation by ouabain and its cardiotoxic actions

The doses of ouabain that caused maximal chemoreceptor discharge are shown in Table 5.1. Comparing these doses with that needed to cause ventricular extrasystoles when administered i.c. (64 ± 5 μg/kg,

n=3) or i.v. (69 ± 2 μg/kg, n=6, Peres-Gomes and Ribeiro, 1979), it is evident that when ouabain is administered i.c. a significant difference ($P < 0.05$) exists between chemoreceptor activation and cardiotoxic doses, whereas little or no difference is apparent between chemoreceptor activation and cardiotoxic effect when ouabain is given i.v.

Evoked responses

Injections (i.c.) of NaCN (5 μg), CO_2-equilibrated Locke solution (0.3 ml), ACh (50 μg) and dopamine (5 μg) were made before and during ouabain infusions (3 μg/min i.c.). The times in Figures 5.8, 5.9, 5.10 and 5.11 at which injections of these substances were performed are approximate, i.e. the injections were made at some time during the 10 min periods over which the chemoreceptor discharge was averaged. Since no substantial differences were detected in the responses evoked by those substances between cats with ganglio-glomerular (sympathetic) nerves cut or intact, responses evoked in both populations were pooled.

Sodium cyanide

The chemoexcitatory responses evoked by NaCN were enhanced during the increase in spontaneous chemoreceptor discharge caused by ouabain and decreased during the phase in which discharge returned to levels recorded before starting ouabain infusion (control) or to levels below control (Figure 5.8).

CO_2-equilibrated Locke solution

An increase in the chemoexcitatory responses evoked by CO_2 was also observed during increase in spontaneous chemoreceptor discharge induced by ouabain and this was followed by a marked

decrease ($P < 0.05$) or even absence of responses to CO_2 during the subsequent decline in chemoreceptor discharge (Figure 5.9).

Acetylcholine

In the case of ACh its chemoexcitatory action was potentiated during the increase in spontaneous chemoreceptor discharge induced by ouabain and decreased during the subsequent decline of the chemoreceptor discharge. However, the decrease in the ACh responses was not so accentuated as in relation to those induced by both NaCN and CO_2 (see Figure 5.10).

Dopamine

The chemoinhibitory action evoked by dopamine was enhanced during the increase in spontaneous chemoreceptor discharge induced by ouabain and was decreased during the subsequent decline of the chemoreceptor discharge observed during ouabain continuous infusion (Figure 5.11).

Adenosine

In the heart, adenosine potentiates the cardiotoxic effects of cardiac glycosides (e.g. Rand, Stafford and Thorp, 1955). In order to investigate whether ouabain influenced the responses to adenosine, injections of submaximal doses of adenosine (20 and 50 µg i.c.) were made during a ouabain infusion (25 µg/min i.c.) and compared with the effect of adenosine (100 µg i.c.) administered before commencing ouabain infusion. Figure 5.12 illustrates the effects of ouabain and the responses evoked by adenosine. Ouabain itself increased chemoreceptor discharge within the usual pattern of the ouabain infusions but the time to reach the peak was decreased (~ 12 min) as compared with the infusions performed with lower concentrations (see e.g. Figure 5.2).

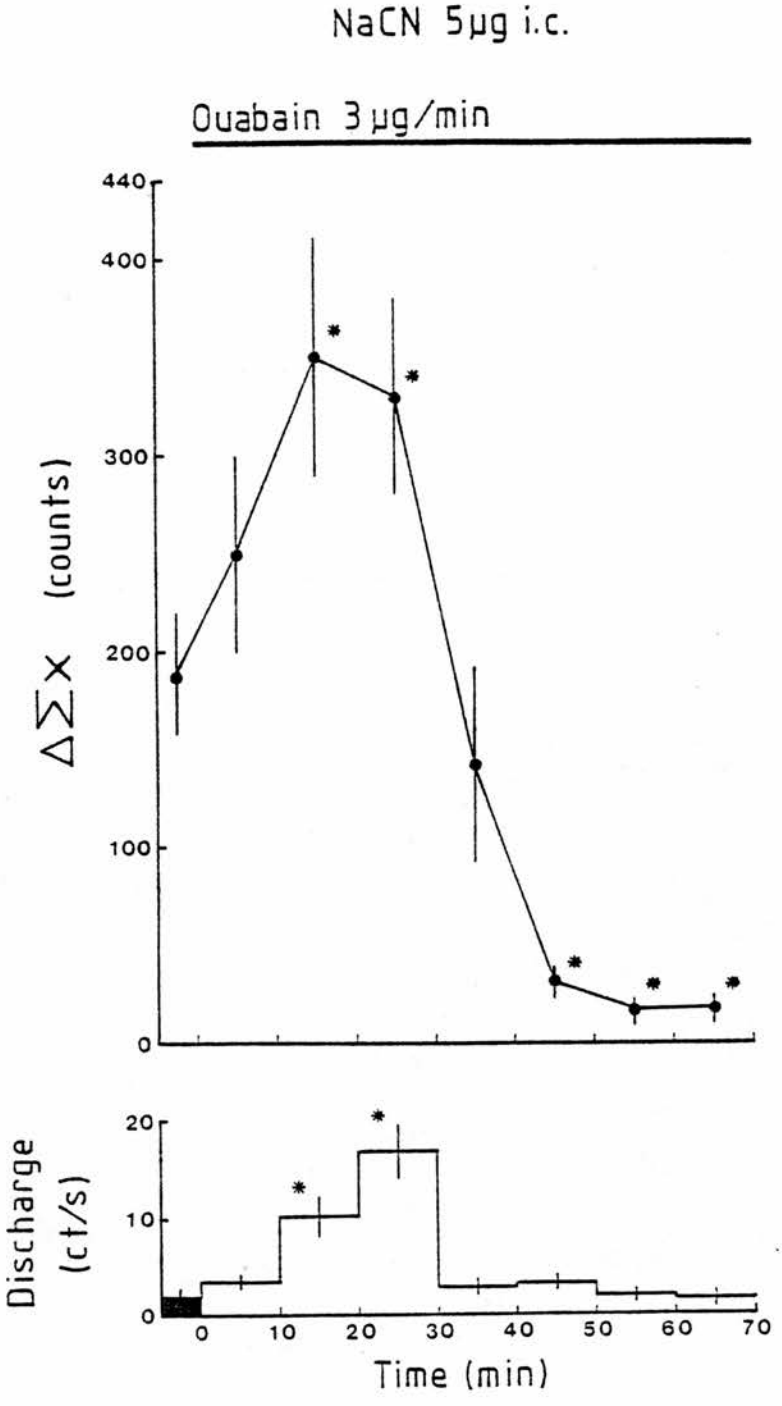


FIGURE 5.8

Effects on chemoreceptor discharge of injections of NaCN (5 µg i.c.) during ouabain infusions (3 µg/min i.c., n=10) performed in five to ten cats. In the lower part the chemoreceptor discharge (ct/s) is shown averaged over 10 min periods during the ouabain infusion. The black rectangle represents averaged chemoreceptor discharge before starting ouabain infusions and the horizontal bar indicates the duration of ouabain infusions. The times given for the injections of NaCN are approximate.

* P <0.05 (t-test)

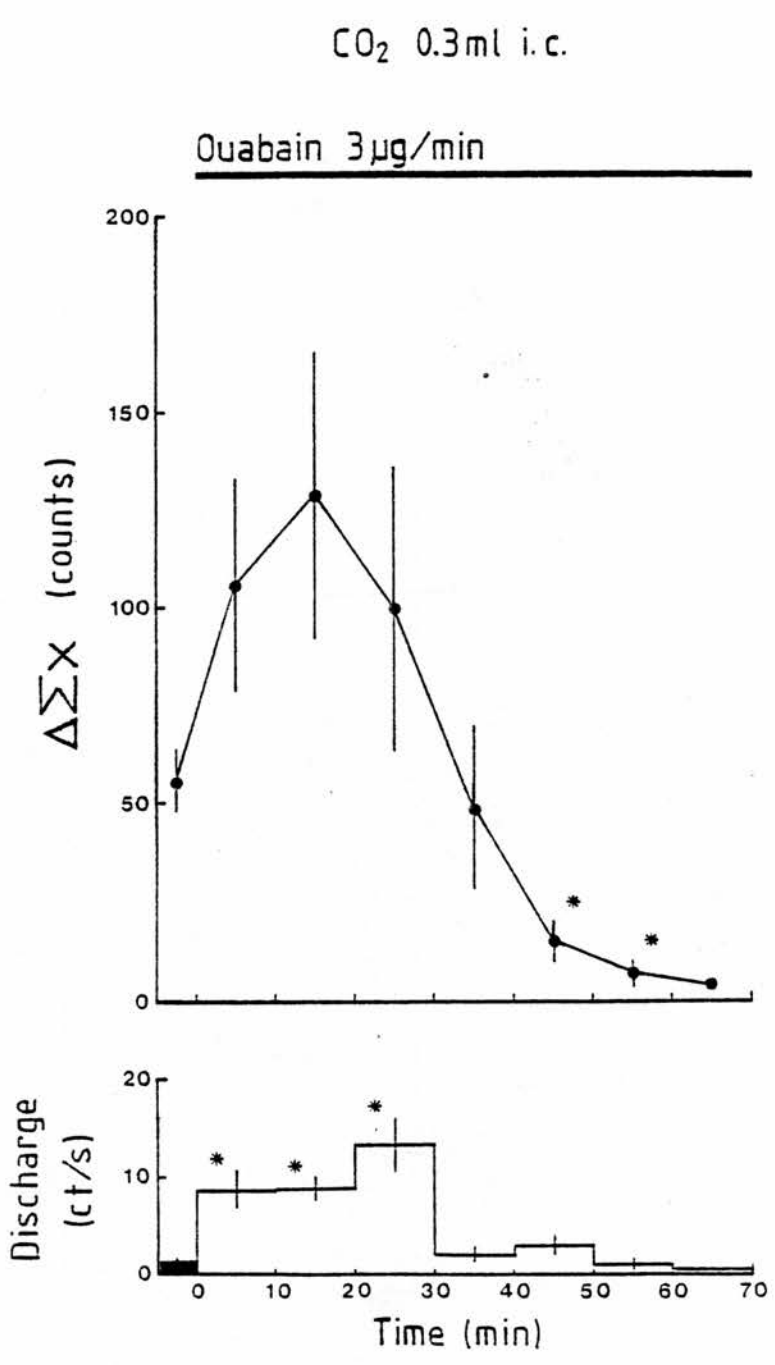


FIGURE 5.9

Effects of injecting CO_2-equilibrated Locke solution (0.3 ml i.c.) during ouabain infusions (3 µg/min i.c., n=8). In the upper part each point is the mean of five to eight experiments. In the lower part the chemoreceptor discharge (ct/s) averaged in 10 min periods during ouabain infusions (3 µg/min i.c., n=8) is shown. The times at which the injections of CO_2 were made are approximate. For further details see legend of Figure 5.8.

* P <0.05 (t-test)

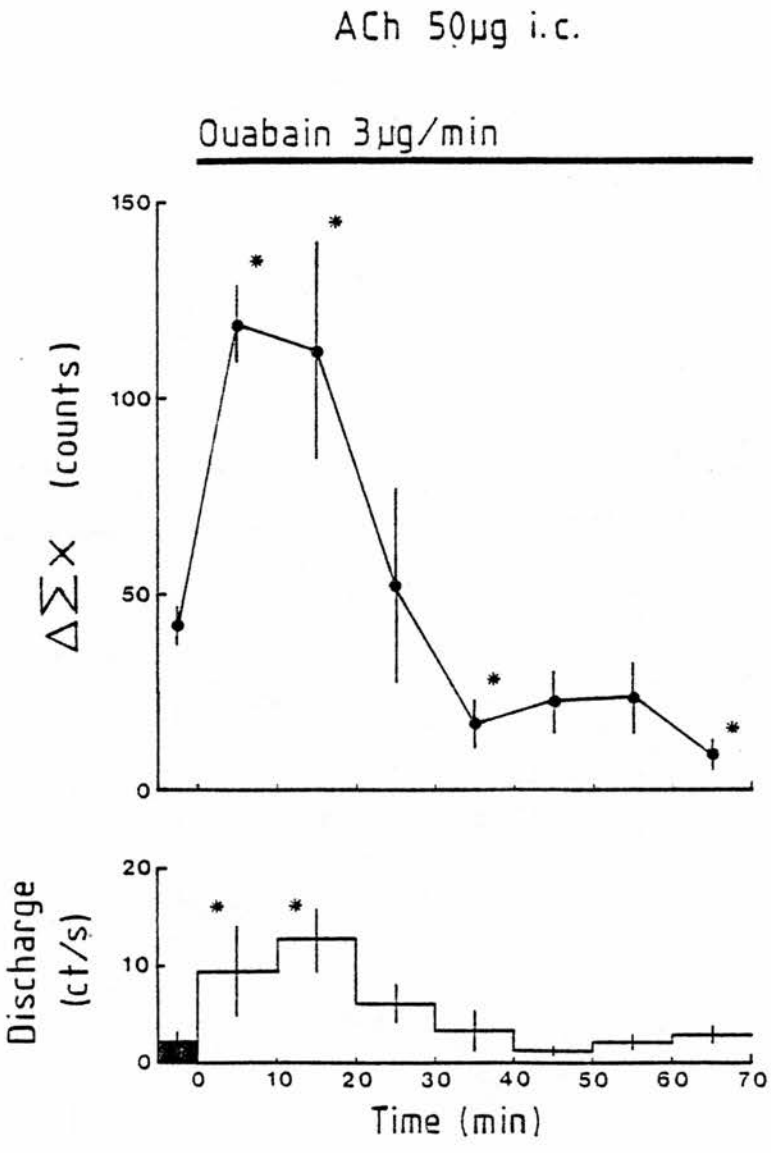


FIGURE 5.10

Effect of injecting ACh (50 µg i.c.) during ouabain infusions (3 µg/min i.c.). In the upper part each point is the mean of five to eight experiments. Note that the responses evoked between 40 and 50 min, and between 50 and 60 min are not significantly different from the control. In the lower part the chemoreceptor discharge (ct/s) averaged in 10 min periods during ouabain infusions (3 µg/min i.c., n=8) is shown. The times at which the injections of ACh were made are approximate. For further details see legend of Figure 5.8.

* $P < 0.05$ (t-test)

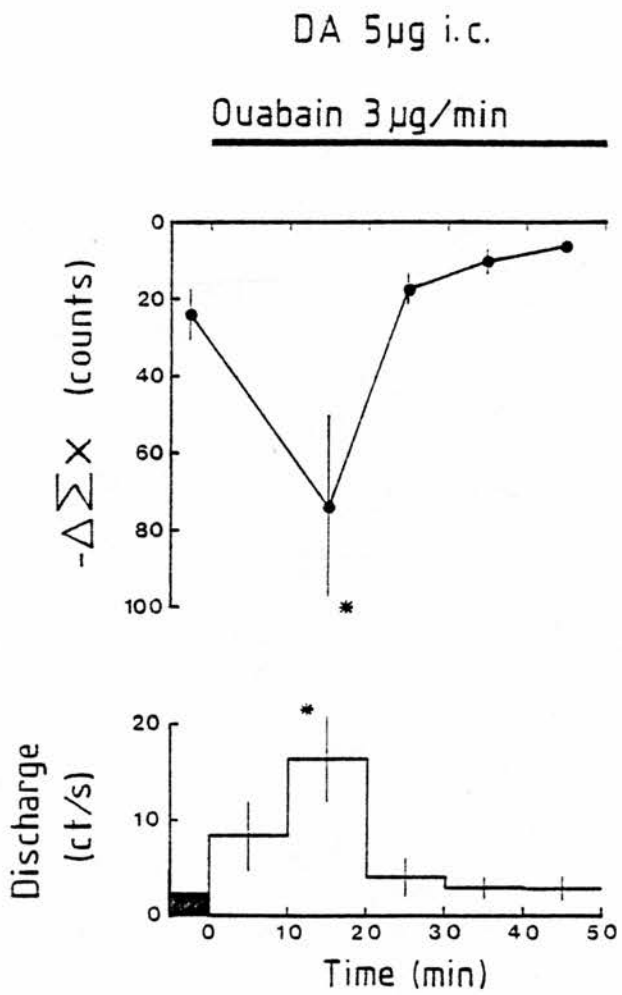


FIGURE 5.11

Effects of injecting dopamine (DA 5 µg i.c.) during ouabain infusions (3 µg/min i.c.). In the upper part each point is the mean of three to five experiments. In the lower part the chemoreceptor discharge (ct/s) averaged in 10 min periods during ouabain infusions (3 µg/min i.c., n=5) is shown. The times at which the injections of DA were made are approximate. For further details see legend of Figure 5.8.

* $P < 0.05$ ($\underline{t}$-test)

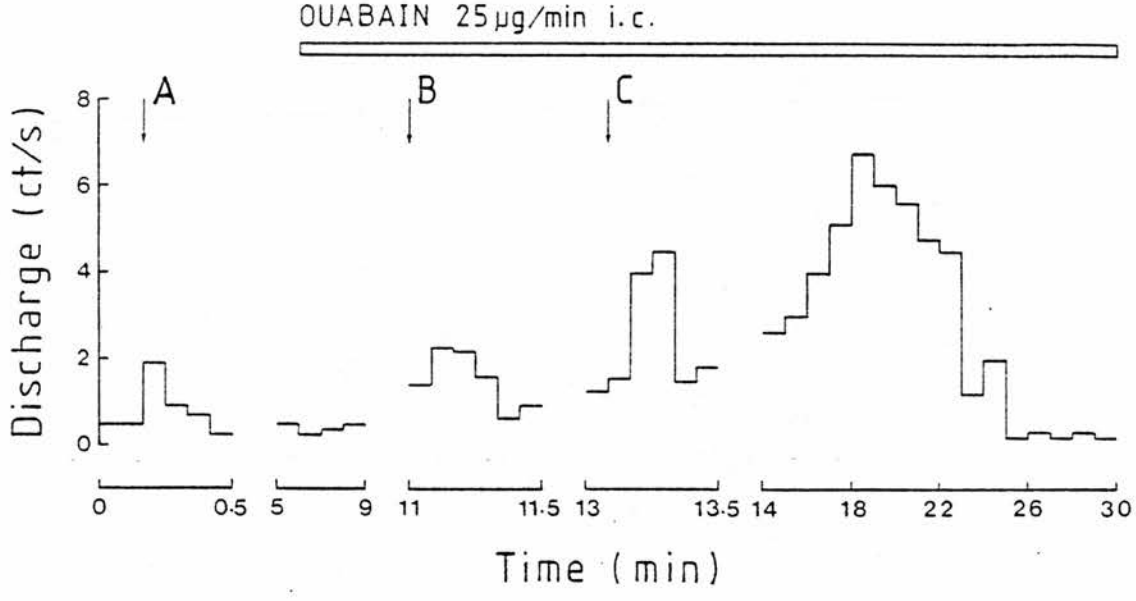


FIGURE 5.12

Effect of adenosine injections (i.c.) on chemoreceptor discharge (ct/s) (recordings obtained from a single chemoreceptor unit) before and during a ouabain infusion (25 µg/min i.c.). A. Response to adenosine (100 µg) before starting ouabain infusion; B. Response to adenosine (20 µg) administered 5 min; and C. Response to adenosine (50 µg) administered 7 min after starting ouabain infusion. Note that the response to adenosine (50 µg) was greater than that obtained with 100 µg before starting ouabain infusion. The arrows indicate the times of injections. The horizontal bar represents the duration of the ouabain infusion.

The response to adenosine (20 µg i.c.) at 5 min and at 7 min (50 µg i.c.) was potentiated (Figure 5.12). No responses were evoked by adenosine during the decline of the response to ouabain infusion.

Leucine-enkephalin

In one experiment leu-ENK (10 - 100 µg i.c.) was administered after a series of successive ouabain injections 1, 10 and 100 µg i.c. The injections of leu-ENK were performed during the increase in chemoreceptor discharge induced by ouabain 100 µg i.c. The injections, 10 µg and 100 µg, were made 5 and 10 min respectively after ouabain injection. Both injections of leu-ENK caused inhibition in the chemoreceptor discharge evoked by ouabain.

This experiment had only a qualitative purpose, that to test if inhibitory responses could still be evoked by the enkephalins in the presence of ouabain.

Dihydro-ouabain

Ouabain in low concentrations stimulates and high concentration inhibits Na^+, K^+-ATPase whereas the ouabain analogue dihydro-ouabain has been considered to be a pure Na^+, K^+-ATPase inhibitor (e.g. Ghysel-Burton and Godfraind, 1979). In order to determine whether the effect or part of the effect of ouabain might be attributed to its Na^+, K^+-ATPase stimulating properties it was decided to investigate the response of chemoreceptors to dihydro-ouabain.

Two experiments were performed, dihydro-ouabain was infused i.c. close to the carotid body at two different rates 3 µg/min i.c. and 9 µg/min i.c. The low dose had little or no effect on chemoreceptor discharge as compared with ouabain (3 µg/min) infusions. However,

when administered at a rate of 9 µg/min i.c. (see Figure 5.13) its activation of chemoreceptor discharge followed a pattern similar to that observed during ouabain infusions.

Physostigmine and mecamylamine

Ouabain releases ACh from both central and peripheral nervous systems (see Section I, General Introduction). In order to investigate whether endogenous ACh would be responsible for the increase in chemoreceptor discharge caused by ouabain, three experiments were designed. In one experiment physostigmine (a potent anticholinesterase, effective against both acetylcholinesterase and pseudocholinesterase; see e.g. Koelle and Gilman, 1949) was used. The effect of ouabain (3 µg/min i.c.) was investigated in a cat pretreated with atropine (1 mg/kg i.v.) and physostigmine (1 mg/kg i.c.) (see Figure 5.14A). Ouabain increased chemoreceptor discharge in a manner similar to that usually obtained, and the increase was followed by a subsequent decline in the discharge.

In another two experiments mecamylamine was used. Mecamylamine is a potent ganglion blocking drug (e.g. Stone, Torchiana, Navarro and Beyer, 1956) which can reduce or abolish the increase in chemoreceptor activity evoked by ACh in the cat carotid body *in vitro* (Eyzaguirre and Zapata, 1968b) or *in vivo* (Sampson, 1971; Nishi and Eyzaguirre, 1971; McQueen, 1977).

In the experiments with mecamylamine, the appropriate volume of dextran solution (25% dextran, 5% glucose in distilled water) required to maintain B.P. at the control level was administered i.v. This treatment prevented the fall in B.P. which would otherwise have accompanied administration of mecamylamine, and hence making the

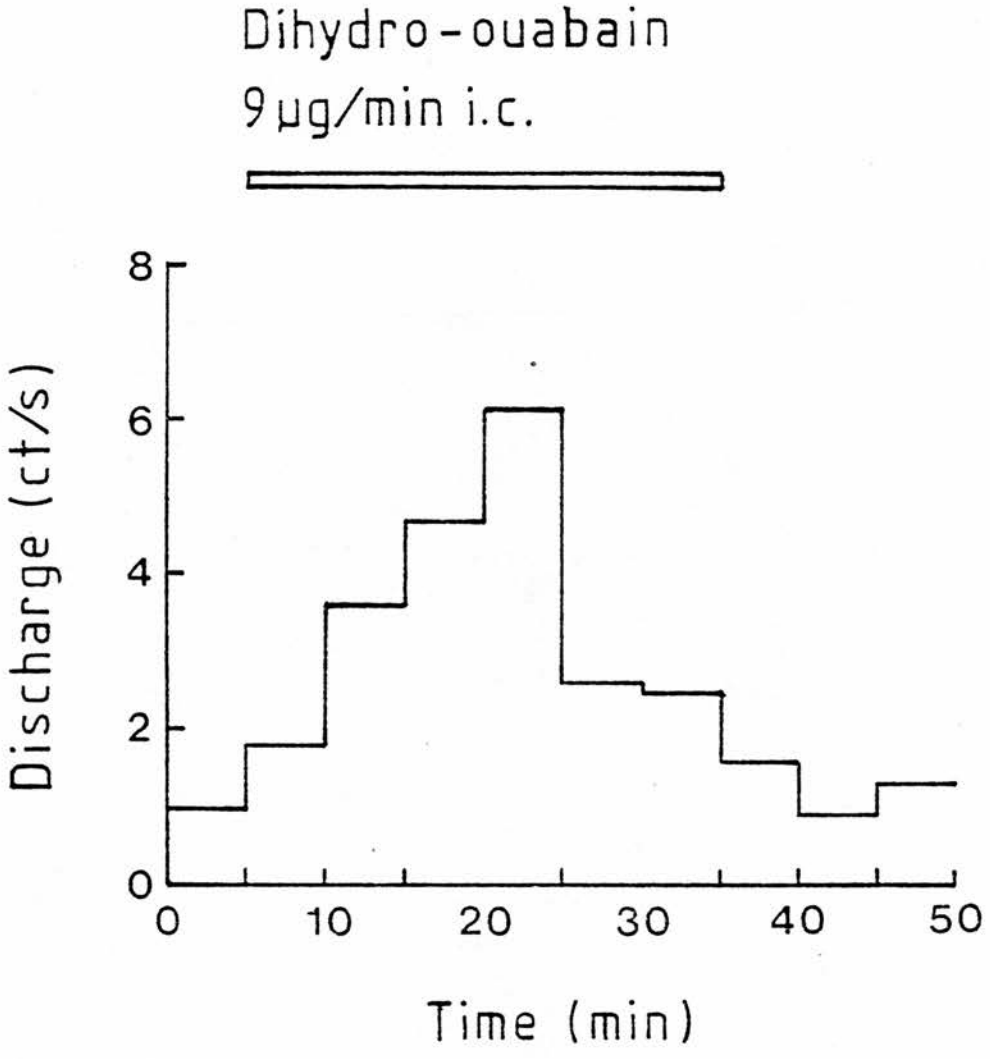


FIGURE 5.13

Effect of infusing dihydro-ouabain (9 µg/min i.c.) on spontaneous chemoreceptor discharge (ct/s), in a cat with the ganglioglomerular (sympathetic) nerves intact. Recordings from 1 to 2 chemoreceptor units. Discharge was averaged over 5 min periods during 50 min. The horizontal bar indicates the duration of the infusion.

interpretation of results difficult, because severe hypotension causes increase in background discharge. Figure 5.14B illustrates one of the experiments performed to explore whether mecamylamine influences the action of ouabain. Mecamylamine in the dose given (5 mg/kg i.c.) was able to antagonize the responses to ACh (25 - 250 µg i.c.), but the chemoexcitatory effect of ouabain and the general pattern of its responses (Figure 5.14B) was similar to that described previously in the absence of mecamylamine (see e.g. Figure 5.3).

α-flupenthixol

In two experiments ouabain (3 µg/min i.c.) was infused after injecting the dopamine antagonist α-flupenthixol in a dose of 200 µg/kg i.c., which was able to antagonize the inhibitory response to dopamine (2.5 µg i.c.) converting it into an excitation. One experiment is illustrated in Figure 5.15, the chemoreceptor response to ouabain peaked earlier and the general picture of the response was attenuated in relation to both its intensity and duration.

DISCUSSION

The results obtained show that ouabain alters activity of the carotid body chemoreceptors. The effect can be divided into two main phases: increase in spontaneous discharge followed by a return of chemoreceptor discharge to or slightly below the pre-injection (control) levels. During the chemoexcitatory phase responses evoked by NaCN, CO_2-equilibrated Locke solution and ACh were increased as was the chemoinhibitory response evoked by dopamine. During the decline that follows the ouabain chemoreceptor excitation the responses evoked by these substances were reduced. Thus it might be concluded that the

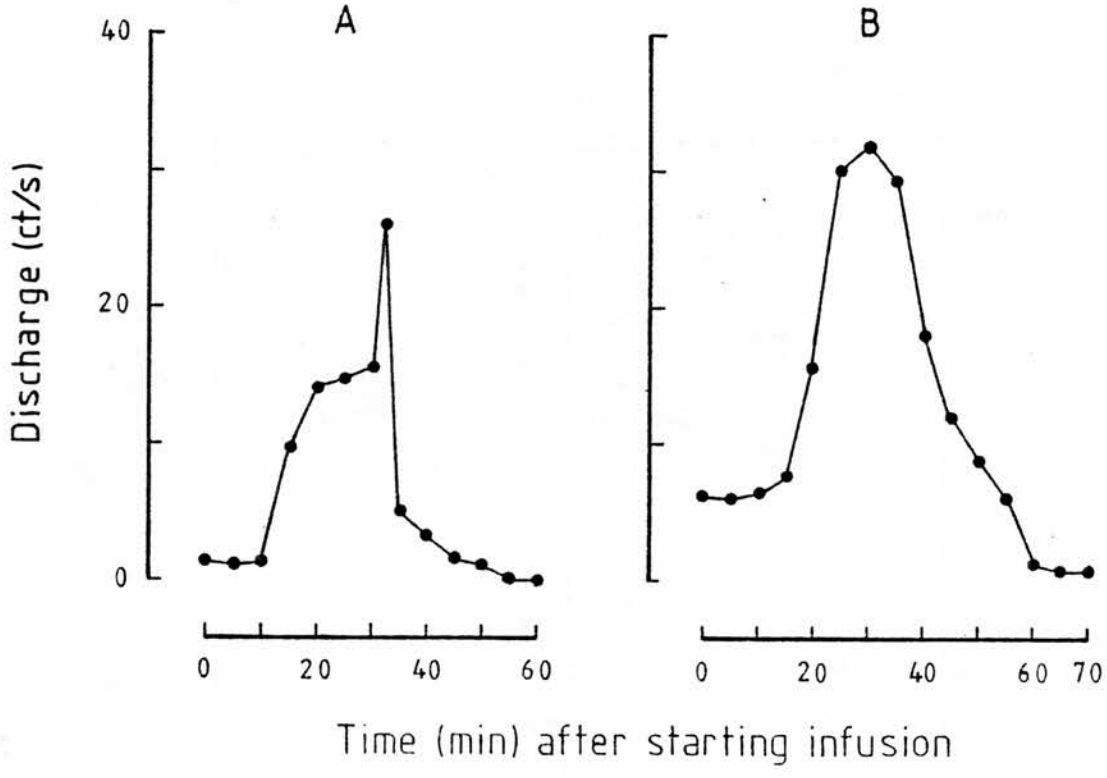


FIGURE 5.14

Effects of ouabain (3 μg/min i.c.) on chemoreceptor discharge in two different cats. A. Response to ouabain infusion after pretreatment with physostigmine (1 mg/kg i.c.). Recordings obtained from 2 to 3 chemoreceptor units. B. Response to ouabain after pretreatment with mecamylamine (5 mg/kg i.v.). Recordings obtained from 4 to 6 chemoreceptor units. The infusion of ouabain started at time o. Dextran was administered i.v. to prevent falls in B.P. occurring following mecamylamine injection.

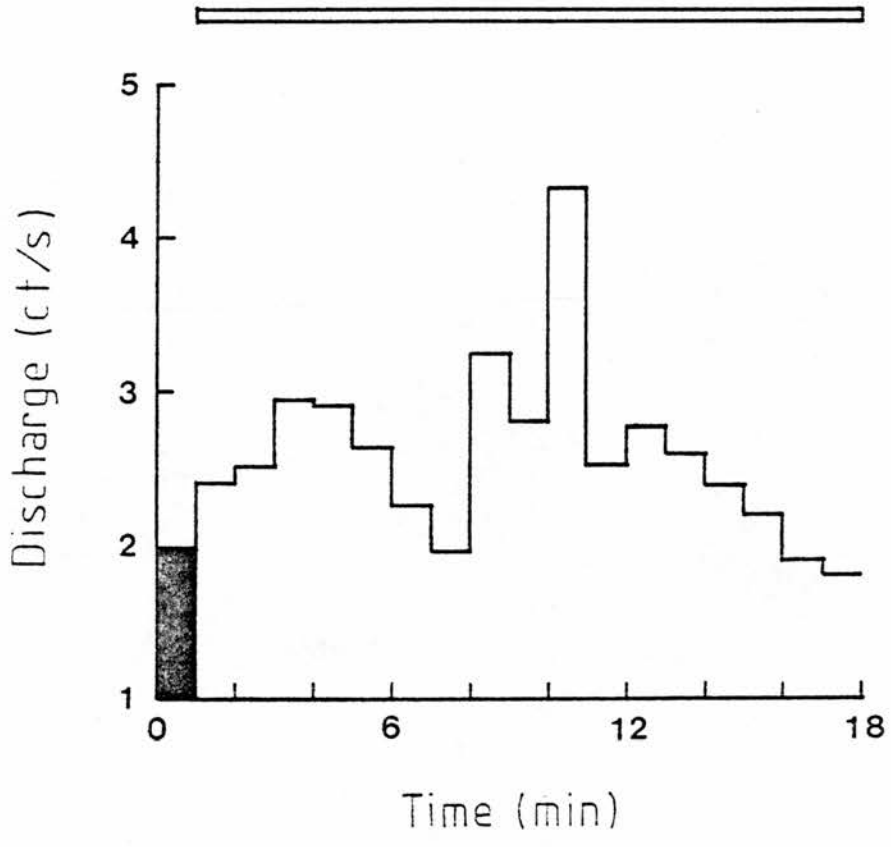


FIGURE 5.15

Effect of ouabain (3 µg/min i.c.) on chemoreceptor discharge (ct/s) after injecting α-flupenthixol (200 µg/kg i.c.). Recordings from two chemoreceptor units. The black rectangle represents the discharge averaged before starting ouabain infusion. The horizontal bar indicates the period during which ouabain was infused.

effect of ouabain on chemoreceptors comprises a 'sensitizing' followed by a 'desensitizing' phase.

The increase observed in chemoexcitatory responses evoked by NaCN, CO_2 or ACh might depend on the increase in background discharge induced by ouabain, but seems probable that at least part of the effect is a consequence of a ouabain-sensitizing process taking place at the carotid body. For example when chemoreceptor discharge returned to the pre-infusion (control) level the evoked responses were greatly diminished and eventually abolished.

As mentioned previously (Section I, General Introduction), a number of authors have reported effects of ouabain on arterial chemoreceptors. McLain (1970) described an increase in the total spontaneous neural traffic (baro- and chemoreceptors) recorded from the cat carotid sinus nerve after injecting ouabain i.v. or i.m. Joels and Neil (1968) found that ouabain inhibits chemoreceptor discharge evoked by perfusion of vascularly isolated cat carotid bodies with either nitrogenated solutions or ACh. Other cardiac glycosides increase spontaneous chemoreceptor activity (Schmitt, Güth and Müller-Limmroth, 1958). The excitation of spontaneous chemoreceptor discharge has been confirmed in the present work. A decrease in the excitatory action of ouabain on chemoreceptor responses evoked by NaCN, CO_2 and ACh was also obtained in the present study during the declining phase that followed the initial increase in chemoreceptor discharge during ouabain infusion. It is not possible to make a direct comparison between the present results and those of Joels and Neil (1968) because, (1) the duration of the ouabain infusions in their experiments is not given, and (2) the experimental conditions were different, which may explain why Joels and Neil (1968) did not report any chemoexcitatory action of ouabain.

The easiest way to explain the present results is in terms of Biscoe's hypothesis (Biscoe, 1971), i.e. the sensory nerve ending behaves as a chemoreceptor. The idea was put forward on the grounds that, "the membrane potential of all nerve cells is determined by ionic concentration gradients that are controlled by the sodium pump. The metabolic energy on which the pump depends for its level of activity is derived from oxygen, in aerobic systems. Thus given an excess of other requirements, the ambient oxygen tension could control the rate of the sodium pump, which in turn would affect the potassium gradient and so the membrane potential. A fall in oxygen tension could slow the pump down, allowing a loss of potassium, which would cause depolarization and which in turn could be sufficient to initiate action potentials in the afferent fiber. With large nerve fibers or cells the effects on the potassium potential would normally be of little account. However, it is possible that because of their very high surface-to-volume ratio the membrane potential of very small fibers will be extremely sensitive to oxygen tension. The electrogenic property of the sodium pump, if taken into account, would reinforce the depolarizing effect of a reduction in pump activity".

It is known that raising the extracellular potassium concentration in the carotid body increases the nervous chemoreceptor activity (Acker, 1978). However, *in vitro* the glomus cell membrane seems to be rather insensitive to changes in extracellular K^+ (Eyzaguirre and Fidone, 1980). Ouabain inhibits the sodium pump (Repke and Portius, 1963). Two phases in the action of ouabain have been postulated. For instance, Baker and Willis (1972) refer the existence of a primary phase that is concentration-dependent but does not follow simple first order kinetics and is a consequence of Na^+, K^+-ATPase inhibition and

a secondary phase that results from uptake of ouabain by nerve endings and its interaction with the movements of Ca^{2+} inside and outside the cells.

In relation to the present results, the 'sensitizing' phase of ouabain would represent inhibition of the Na^+, K^+-pump and the 'desensitizing' phase could be related to paralysis of the Na^+, K^+-pump. The direct sensory nerve ending activation hypothesis is supported by the observation that in the 'sensitizing' phase responses to both ACh and dopamine were potentiated. Furthermore, direct excitation of carotid body baroreceptors by ouabain has been suggested by Quest and Gillis (1974) and most of the arguments used to explain how ouabain causes such activation, i.e. by reducing the threshold for excitation of sensory receptors in particular those related to C fibres, could also apply to the present findings. However, afferent chemoreceptor activity seems to be conducted by one third of C fibres and two thirds of A fibres (Fidone and Sato, 1969), making more probable that the present recordings were obtained from A fibres. Whether the ouabain effect differs according to the fibre affected cannot be determined from the present observations.

The difficulty in obtaining full recovery after ouabain is probably related to its mechanism of action and binding of ouabain. Assuming that Na^+, K^+-ATPase inhibition is responsible for its excitatory action after using high doses, it might be accepted that what has been described for other tissues may also be applied to the present findings. The ouabain-Na^+, K^+-ATPase complex is extremely stable (Peters, Raben and Wassermann, 1974). It cannot be dissociated within 30 min.

The decay phase of the ouabain response does not seem to result from an exhaustion mechanism because mimicking the pattern of the

ouabain response by applying first partial hypoxia (10% O_2 + 90% N_2) and then severe hypoxia (100% N_2) did not prevent further chemoreceptor responses (Figure 5.6). However, one could argue that higher doses of ouabain paralyse the Na^+, K^+-ATPase. This could trigger a biochemical mechanism, which eventually might exhaust the mechanisms involved in the increased discharge evoked by ouabain.

For example, Fidone, Gonzalez and Yoshizaki (1981) described that in the rabbit superfusing the carotid body *in vitro* with no Ca^{2+} and high Mg^{2+} a nearly complete block of ^3H-dopamine release occurred, yet only about a 50% reduction in nervous activity was observed.

The carotid body type I cell has been classified as being in the APUD cell series (Pearse, 1969) and within the paraneurone group (Kobayashi, 1977) (see Section I, General Introduction). It contains amines, peptides and ATP (see e.g. Fujita and Kobayashi, 1979). The amine so far identified and being an effective depressant at the carotid body is dopamine (for a review see Fidone, Gonzalez and Yoshizaki, 1980). The peptide-like substances so far identified as being present are met-ENK, leu-ENK, SP and VIP. The enkephalins depress chemoreceptor discharge (see Section IV); SP causes a delayed increase in discharge (McQueen, 1980) and VIP in low doses depresses and in high doses increases chemoreceptor discharge (see Section IV). ATP and its closely related nucleoside, adenosine, increase chemoreceptor discharge (see Section III). Classifying the actions of all those substances according to speed of onset and duration of the response it is possible to distinguish two groups: one with rapid onset (1 to 5 s) and a short-lasting effect, namely the enkephalins and adenosine, and another with delayed onset and a long lasting effect exemplified by SP and VIP. The latter, appear to have properties usually associated with neurohormones and the former group more those of neurotransmitters.

It is well documented that ouabain can release the classical neurotransmitters (ACh and NA) both *in vitro* (for reviews see Vizi, 1979; Gillis and Quest, 1980) and *in vivo* (Ribeiro and Peres-Gomes, 1977) as well as adenosine (Shimizu, Creveling and Daly, 1970; Daval, Barberis and Gayet, 1980; Hollins and Stone, 1980a; Hollins, Stone and Lloyd, 1980). Thus the possibility of ouabain releasing all these substances (i.e. 'classical' neurotransmitters, peptides, adenosine) from the carotid body has to be considered, meaning that its effect might result from release of inhibitory and excitatory neurotransmitters or neuromodulators in the carotid body. From the present results it is not possible to establish the importance of these factors. For example, ACh appears not to be any more important than other stimulatory influences since no substantial changes were seen in the pattern of the ouabain response either after physostigmine or mecamylamine. Dopamine might be involved because in the cats pretreated with the dopamine antagonist α-flupenthixol, ouabain caused a less exuberant increase in chemoreceptor discharge and the peak occurred earlier. Sensitization to exogenous ACh, dopamine and adenosine was also detected. Whether these actions mean involvement of the same endogenous substances as well as those of peptides deserve further investigation. In the case of adenosine, it will be necessary to determine the effect of ouabain in the presence of an adenosine uptake blocker (e.g. dipyridamole) or after pretreatment with adenosine deaminase inhibitors such as deoxycoformycin. In the case of the enkephalins it would be interesting to know whether naloxone modifies the response to ouabain. The chemoinhibition induced by leu-ENK is still present during the ouabain excitatory phase. Interactions with other peptides also present in the carotid body such as SP and VIP might also be of interest.

An efferent pathway has been postulated (see Biscoe, 1971; Osborne and Butler, 1975) so ouabain might interfere with the release of substances involved in that pathway. What transmitters are released or how ouabain affects this component is impossible to determine as its physiological relevance has not yet been established (but see Section 1, General Introduction).

The metabolic inhibitors have amongst other actions, the ability to inhibit the sodium pump. For instance, at the neuromuscular junction 2-4-dinitrophenol (Beani, Bianchi and Ledda, 1966) or hypoxia (nitrogen atmosphere) (Hubbard and Løyning, 1966) increase the frequency of miniature end-plate potentials in a manner similar to that caused by ouabain. The same is in general observed with procedures that interfere with the regeneration of ATP. Although in the present study ouabain appears to mimick, at least in part, the hypoxic response, striking differences were noted: (1) the response of chemoreceptors to hypoxic stimulation can be maintained throughout the period of stimulation, whereas during ouabain infusion chemoreceptor discharge begins to fall shortly after peak discharge is reached; (2) after hypoxic stimulation is stopped and discharge returned to the pre-infusion levels responses evoked by substances such as NaCN, CO_2, ACh and dopamine are not substantially altered, whereas after ouabain infusion that lasted about the same time as hypoxic stimulation the responses to those stimulants were markedly reduced or absent. A reason for these differences may be, as Birks and Cohen (1968) pointed out for the neuromuscular junction, from ouabain acting specifically on the Na^+, K^+-ATPase which behaves as the ouabain receptor, whilst the metabolic inhibitors and/or hypoxia act indirectly by interfering with the synthesis of ATP.

The persistence of chemoreceptor discharge after death seems to indicate that part of the chemoreceptor discharge is independent, at least for 10 to 40 min, of a number of factors such as the profound change in B.P., alterations in arterial blood gases (PaO_2, $PaCO_2$) and pH, as well as becoming unresponsive to the 'classical' chemoreceptor stimulants NaCN, CO_2 and ACh. Alternatively the absence of chemoreceptor response might result from the chemoreceptors adapting to different stimuli in consequence of ouabain action. According to Nakajima and Onodera (1969) an electrogenic sodium pump could be responsible for adaptation processes at the sensory receptors. An adaptation to hypercapnia has been reported by McCloskey (1968) and Gray (1968). Thus, the persistence of the discharge after cardiac arrest might correspond to the existence of an interaction with an adaptative process.

Another possibility is that a metabolite capable of activating chemoreceptors is being released, by whatever leakage process, after death. For instance, at the central nervous system, *in vivo*, Ribeiro and Peres-Gomes (1977) were able to detect by bioassay a considerable output of an ACh-like substance, until 60 min after cardiac arrest, in the cerebrospinal fluid. The appearance of ACh seems to be independent of the cause that induced cardiac arrest (e.g. ouabain infusion i.v. or intracerebroventricular; electrical stimulation, aconitine or potassium chloride directly applied to the external surface of the cardiac ventricles). If the same applies to the carotid body then one could revive the metabolite hypothesis advanced by Neil (1951) and Landgren and Neil (1951), which had its origin with Bogue and Stella (1935) on the grounds of similar observations to those presently described, i.e. the prolongation of the chemoreceptor discharge after

death. This hypothesis, however, has been refuted by Daly, Lambertsen and Schweitzer (1954) and Paintal (1967) on the grounds that chemoreceptors are highly sensitive to falls in O_2 and that discharge of chemoreceptors falls within 2 to 3 min after circulatory arrest (see e.g. Paintal, 1971).

In conclusion, ouabain affects chemoreceptor activity first increasing spontaneous chemoreceptor discharge and sensitizing chemoreceptors to the action of substances such as NaCN, CO_2, ACh, dopamine and adenosine: this can be considered a 'sensitizing' phase. Secondly, during the decline of chemoreceptor discharge that follows the 'sensitizing' phase ouabain alters chemoreceptor activity in a manner that the chemoreceptors usually become much less responsive or even unresponsive to ouabain itself, NaCN, CO_2, ACh, dopamine and adenosine and this can be named 'desensitizing' phase. As ouabain inhibits Na^+, K^+-ATPase and it is likely that the structural components of the carotid body contain that ATPase, the ouabain effect is perhaps the net effect on all the components, i.e. on the glomus type I and type II cells, sensory nerve endings and nerve endings with motor-like function. Sympathetic nerve endings appear to be affected as can be concluded from differences observed between cats with ganglioglomerular (sympathetic) nerves intact and cut during i.c. infusions. Sensory nerve endings, in particular those originating from C fibres should also be activated. Whether the transmitters or any other substances released by ouabain from type I cells should also be considered in the ouabain effect is at present impossible to know.

SECTION VI

General Discussion and Conclusions

I. The results obtained with purines and peptides suggest the following main conclusions:

1. Substances present in the cat carotid body such as ATP/adenosine and the peptides, met-ENK, leu-ENK, and VIP are effective when injected close arterial to the carotid body. These substances can be grouped with others which are also present in the carotid body and also affect chemosensory discharge (e.g. catecholamines, 5-HT, ACh, SP).

2. The effect these substances produced when injected i.c. can be divided on the basis of the speed of onset and duration of the responses into (a) rapid onset and short-lasting (adenosine and the enkephalins) and (b) slower onset and long-lasting (VIP). Both adenosine and the enkephalins caused quite clear effects, the former activated the chemoreceptors and the latter inhibited them. The effects of β-endorphin, VIP and CCK-8 were not so clear cut and tended to be biphasic.

3. Considering those substances in molecular terms, adenosine and the enkephalins are relatively small molecules compared with β-endorphin, VIP or CCK-8. In this respect adenosine and the enkephalins are closer to neurotransmitters and VIP, β-endorphin and CCK-8 to neurohormones.

4. The effects on chemosensory discharge of the different substances present in the cat carotid body following i.c. injection in the cat can be distinguished into: (a) inhibitory, obtained with dopamine and the enkephalins; (b) excitatory, obtained with ACh and ATP/adenosine; (c) biphasic, obtained with VIP and SP.

5. β-endorphin and CCK-8 are present in various parts of the body but it is not yet established whether or not they are present in the carotid body, and have biphasic actions on chemoreceptor activity.

II. Another important conclusion relates to ouabain, which can both excite and inhibit chemoreceptor activity. Two phases can be detected, a 'sensitization' followed by a 'desensitization' phase. These effects are probably related to the well-established properties of ouabain in various tissues, namely its ability to modify Na^+, K^+-ATPase.

Some of the substances mentioned above have been suggested as being involved in sensory transmission. For example, ATP was suggested as a sensory transmitter of both central and peripheral sensory nerve endings (Holton and Holton, 1953; 1954). The enkephalins may be the endogenous ligands for the opiate receptor and as such they have been considered as participants in the analgesia processes (see e.g. Kosterlitz and Hughes, 1975). SP has been frequently proposed as a sensory (? pain) transmitter (see e.g. Nicoll, Sehenker and Leeman, 1980).

Interactions between the enkephalins and SP and between VIP and ACh or in general terms between peptides and the 'classical' transmitters as well as between ATP/adenosine and the 'classical' transmitters have been considered in the recent literature in order to explain both central and peripheral excitation and/or inhibition. Thus, electrophysiological evidence is compatible with three actions of opioid peptides and adenosine at the present time. Firstly, there is a presynaptic action to depress the release of certain neurotransmitters (a neuromodulator effect). The mechanism underlying this might involve the

decrease in uptake of calcium ions by the nerve endings. Secondly, opioid peptides and adenosine can interfere directly with the post-synaptic actions of certain neurotransmitters, altering their ability to change ionic conductances (a cotransmitter effect). The third action is a direct 'postsynaptic' action on some cells (a neurotransmitter effect). When this action occurs, as in the case of the opioid peptides, it may be predominantly inhibitory. The present evidence, however, does not allow one to conclude which one of these is the most important action underlying the acute effects of the purines or opioid peptides on the activity of the carotid body chemoreceptors.

The opioid peptides may be divided into two independent groups which act in different systems (see Kosterlitz, 1979). The first is represented by the short-chain peptides met-ENK and leu-ENK and they are distributed unevenly throughout the brain, spinal cord and peripheral nervous system (e.g. Elde, Hökfelt, Johansson and Terenius, 1976; Hughes, Kosterlitz and Smith, 1977). The second group is represented by the long-chain peptide β-endorphin, present in the hypothalamus-pituitary axis and extending into the midline regions of the diencephalon and anterior pons (e.g. Bloom, Rossier, Battenberg, Bayon, French, Henriksen, Siggins, Segal, Browne, Ling and Guillemin, 1978; Watson, Barchas and Li, 1977). On this basis, according to Kosterlitz (1979), only the enkephalins should be considered as neurotransmitters in the peripheral nervous system. In certain stress conditions β-endorphin may be released into the blood stream, but its concentration is unlikely to be high enough to affect peripheral tissues; if such an effect were to occur, it should be classified as a hormonal action (see Kosterlitz, 1979).

The evidence that adenosine is a neurotransmitter in the brain is still unclear and the fact that it has multiple metabolic roles make it

difficult to identify a potential neurotransmitter function for this substance (see e.g. Snyder, Bruns, Daly and Innis, 1981). However, adenosine has important roles in many functions of the central and peripheral nervous systems. The electrophysiological expression of the changes it produces is a reduction of spontaneous and evoked neuronal activity (see e.g. Ribeiro, 1979) and the biochemical expression is the inhibition of adenylate cyclase via a 'high' affinity receptor and its excitation via a 'low' affinity receptor. The first is localized intracellularly and the latter extracellularly (see e.g. Daly, Bruns and Snyder, 1981; Stone, 1981).

On the other hand, adenosine is released into the circulation by a number of physiological and pathophysiological processes (e.g. ischaemia; see Winn, Rubio and Berne, 1979), and a correlation between adenosine concentration and oxygen supply in rat brain has been suggested (Rubio, Berne, Bockman and Curnish, 1975). In the present work low doses of adenosine modified chemoreceptor discharge, and it seems reasonable to speculate that adenosine might influence events taking place in the carotid body by acting as a neuromodulator, cotransmitter or neurotransmitter.

In cells such as those in the adrenal medulla and the SIF cells, which have several similarities to those of the carotid body type I cells ATP, peptides and classical transmitters coexist, can be released together, and may interact postsynaptically (e.g. Burnstock, Hökfelt, Gershon, Iversen, Kosterlitz and Szurszewski, 1979; Viveros, Diliberto, Hazum and Chang, 1979; Wilson, Klein, Chang, Gasparis, Viveros and Yang, 1980; Costa, Guidotti, Hanbauer, Hexum, Saiani, Stive and Yang, 1981). If the same occurs in the carotid body their physiological action is probably composite. However, at present it is not possible to

ascertain in which of the carotid body structures these substances act, i.e. type I cells, type II cells, sensory nerve endings, efferent terminals, or whether all these components are influenced through postsynaptic or presynaptic receptors. In physiological conditions the sympathetic nerve endings that regulate carotid body flow may also be affected.

The classical pharmacological criteria, based on the notion of specific drug receptors, namely supported by the use of specific agonists and antagonists, appear to apply particularly to the enkephalins which act through a naloxone-sensitive receptor. Theophylline in the doses studied did not behave as an adenosine antagonist. No specific antagonists are available for the other neuropeptides. It is generally recognised that antagonists are necessary to characterize the receptor, but in the absence of specific antagonists much emphasis has recently been put on radioligand binding studies at neurotransmitter receptors since they can provide discrimination at the molecular level, permitting the differentiation of multiple receptor subtypes (see e.g. Daly, Bruns and Snyder, 1981; Snyder, Bruns, Daly and Innis, 1981).

The vascular effect

The fact that the neuropeptides studied so far, as well as adenosine (for adenosine see also Discussion in Section III), can affect the vasculature could be taken to imply that the role of endogenous carotid body peptides and adenosine is to modify blood flow. However, it is not known whether endogenous peptides or adenosine do actually change carotid body flow, and 'classical' neurotransmitters, such as ACh, noradrenaline and dopamine, have marked effects on blood vessels when injected. This has not prevented these latter substances from being considered as putative neurotransmitters in the carotid body (see e.g.

Biscoe, 1971). So, by the same token the vascular effects caused by exogenously applied neuropeptides or adenosine should not exclude them from consideration as putative neurotransmitters in the carotid body.

Ouabain also affects the vasculature and, hence, some of the above arguments could also apply. But the fact that this substance affects neural tissues implies that in its action on chemoreceptors other mechanisms are involved, namely those related to changes in transmitter output(s) and nerve depolarization.

Cyclic AMP

Despite the presence of various substances in the carotid body affecting different receptors, they may eventually influence a common mechanism (e.g. adenylate cyclase) within the carotid body. This common mechanism can be affected by drugs such as ouabain or by potassium, electrical depolarization, anoxia. The involvement of cyclic AMP is supported by the presence in the carotid body sensory nerve endings of an adenylate cyclase-cyclic AMP system (Fitzgerald, Rogus and Dehghani, 1977) and the findings that isoprenaline stimulates cyclic AMP formation in the rat carotid body *in vivo* (Mir, Pallot and Nahorski, 1981) and stimulates spontaneous chemoreceptor discharge through β-receptors (Folgering, Ponte and Purves, 1980).

A brief discussion postulating the involvement of cyclic AMP in both activation and inhibition of chemoreceptor activity by the substances investigated in the present work follows.

It is now well-established that cyclic AMP formed from ATP by activation of the membrane-bound enzyme adenylate cyclase serves as an intracellular messenger for the action of a number of circulating hormones and neurotransmitters (for reviews see e.g. Greengard, 1976; Daly, 1977; Nathanson, 1977). The enzymatic machinery involved in

the process of cyclic AMP formation includes: adenylate cyclase, cyclic nucleotide phosphodiesterase, cyclic AMP-dependent protein kinase, phosphoprotein phosphatase, and the protein substrates for phosphorylation (see Nathanson, 1977). The mechanism of action of cyclic AMP in neuronal function involves the protein phosphorylation which then regulates permeability to calcium (see e.g. Greengard, 1976).

Adenosine

Adenosine causes a great increase in cyclic AMP through two mechanisms (1) by its incorporation into ATP thus increasing the concentration of substrate available to adenylate cyclase (Shimizu and Daly, 1970; Skolnick and Daly, 1975) and (2) through activation of a specialized adenosine receptor linked with adenylate cyclase (e.g. Huang and Daly, 1974; Londos and Wolff, 1977).

Dopamine

A dopamine-sensitive adenylate cyclase has been identified in several mammalian nerve tissues including sympathetic ganglia (Kebabian and Greengard, 1971) and other parts of the nervous system (central and peripheral) as well as an adenylate cyclase-sensitive to noradrenaline (see Nathanson, 1977).

The fact that sulpiride (a dopamine D_2 antagonist) can antagonize the chemoinhibitory action of dopamine (McQueen, personal communication) suggests the presence of D_2 dopamine receptor which operates without increasing the concentration of cyclic AMP (see e.g. Tsuruta, Frey, Grewe, Cote, Eskay and Kebabian, 1981). However, whether such a receptor exists in the carotid body remains to be established.

Morphine and the enkephalins

Morphine inhibits stimulation of adenylate cyclase by prostaglandins in the brain (Collier and Roy, 1974) and this effect is antagonized by naloxone. The same can also apply to the endogenous ligands for morphine, the enkephalins (see Nathanson, 1977).

Substance P

Adenylate cyclase activity stimulated by SP has been found in both rat and human-brain (Duffy, Wong and Powell, 1975).

Cholecystokinin

In some systems CCK may enhance adenylate cyclase. However, in the pancreas it does not stimulate cyclic AMP formation even though cyclic AMP derivatives do mimic the ability of CCK to enhance amylase secretion (see e.g. Snyder, Bruns, Daly and Innis, 1981).

Vasoactive Intestinal Polypeptide

This peptide also stimulates adenylate cyclase and secretion in several tissues such as intestinal smooth muscle (see e.g. Said, 1978).

Anoxia

Anoxia increases cyclic AMP and cyclic GMP (Kimura, Thomas and Murad, 1974) and this might be achieved via release of adenosine because theophylline antagonizes the increase caused by anoxia (see Daly, 1977).

Ouabain

Ouabain and potassium increase both cyclic AMP and cyclic GMP in the brain (Ferrendelli, Kinscherf and Chang, 1973; Kinscherf, Chang, Rubin, Schneider and Ferrendelli, 1976).

Some of the above substances modify the concentration of cyclic AMP by interfering with adenylate cyclase. Other substances which also change the concentration of cyclic AMP, but by interfering with the phosphodiesterases, such as theophylline and imidazole, do not show such a clear cut correlation between pharmacological and biochemical effects (see e.g. Ginsborg and House, 1980). This might suggest that it is the adenylate cyclase-cyclic AMP system that has to be affected rather than simply the concentration of cyclic AMP.

Table 6.1 correlates presence of substances in the carotid body with chemoreceptor activity and adenylate cyclase changes.

TABLE 6.1: Substances present in the cat carotid body and their effects on chemoreceptor discharge : correlation with their capacity to change the adenylate cyclase-cyclic AMP system

	Effect on the cat Chemoreceptor Discharge	Effect on the Adenylate Cyclase-Cyclic AMP system
Dopamine	↓ ⇡	↓
Noradrenaline	⇣ ↑	↑
ACh	↑	
ATP/adenosine	↑	↑
met-ENK	↓	↓
leu-ENK	↓	↓
SP	⇣ ↑	↑
VIP	⇣ ↑	↑

↓ Decrease

↑ Increase

Broken arrow means slight decrease (⇣) or increase (⇡)

The analysis of the above information suggests that in the cat carotid body, stimulation of chemoreceptor activity results from prior activation of an adenylate cyclase-cyclic AMP system, and inhibition of this system leads to chemosensory inhibition.

Five criteria are generally regarded as necessary for establishing a substance as a neurotransmitter namely: (1) synthesis and storage of transmitter in nerve terminals; (2) release of transmitter during nerve stimulation; (3) postjunctional responses to exogenous transmitter should mimic responses to nerve stimulation; (4) enzymes that inactivate the transmitter and/or an uptake system for the transmitter or its breakdown products; (5) antagonists should block and ag-onists potentiate the responses to both exogenous transmitter and nerve stimulation.

This list is, indeed, enormous compared with the progress made, i.e. only requirements (1) and (3) are closer of being fulfilled in the case of the carotid body. A more 'reasonable' requirement (Werman, 1980) is that at least two criteria have to be met, namely 'collectibility' (the proof of the release of a given material in appropriate concentrations on stimulation of the input nerve) and the criterion of 'identity of action' [(the proof that the action of the substance on the postsynaptic structure - nerve, muscle or gland cell) when present in the extracellular space in sufficient concentration is identical with that of the physiological released material].

As a final comment, from studies on inputs (drugs) and outputs (chemoreceptor discharge) attempts have been made to interpret what happens in the 'black box' (the carotid body). As Crick (1979) pointed out recently "the difficulty with the black-box approach is that unless the box is inherently very simple", which is not the case with the carotid

body, "a stage is soon reached when several rival theories all explain the observed results equally well" (see e.g. the hypotheses listed by Biscoe, 1976). "Attempts to decide among them often prove unsuccessful because as more experiments are done more complexities are revealed." For example, until 1979/80 the carotid body was considered to contain mainly amines (dopamine, noradrenaline, adrenaline, 5-HT) and ACh, but after 1979/80 it was also shown to contain ATP stored with amines, met-ENK, leu-ENK, SP, VIP. As Crick (1979) states, "at this point there is no choice but to poke inside the box if the matter is to be settled one way or the other". So, more extensive studies are needed using more sophisticated methods which have proved useful in other systems, such as microelectrophysiological techniques which certainly help to provide accurate information concerning cellular function and the mechanisms involved, i.e. to know more about pumps, ion channels, drug receptors, enzymes and structural proteins of the cell membranes of the carotid body components.

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APPENDIX I

Publications

[*From the Proceedings of the Physiological Society*, 15–16 *February* 1980
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The effects of ouabain on carotid chemoreceptor activity in the cat

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It is not clear whether ouabain increases (e.g. McLain, 1970) or decreases (Joels & Neil, 1968) chemoreceptor activity, so the present study was undertaken to investigate further its effects on the cat carotid chemoreceptors.

Experiments were performed on pentobarbitone-anaesthetized cats which were artificially ventilated and paralysed with gallamine (3 mg kg^{-1} I.V.). Chemoreceptor activity was recorded from the peripheral end of a sectioned sinus nerve and quantified (McQueen, 1977); the ganglioglomerular (sympathetic) nerves were left intact. Drugs were dissolved in Locke solution and either injected or infused into the ipsilateral common carotid artery (I.C.).

Ouabain was given either as successive injections (0·625, 1·25, 2·5, 5, 10, 20, 40, 80 μg in volumes of 0·1 ml. I.C., every 20–25 min) or as a continuous infusion (3 μg in 0·1 ml. min^{-1} I.C.). Its effect could be divided into two components, an initial excitation followed by a decrease in chemoreceptor activity. *Injections* of ouabain caused a dose-related increase in discharge lasting 1–2 min and a gradual increase in spontaneous discharge. When about 80 μg had been injected spontaneous discharge began to decrease, sometimes very rapidly, and further injections of ouabain were less effective; ultimately discharge ceased entirely. *Infusions* of ouabain caused a gradual increase in discharge frequency until about 20–30 μg had been infused when discharge began to decrease, eventually ceasing. The depression lasted for at least 2–3 hr after stopping the infusion.

Responses to the stimulants NaCN (5 μg I.C. or 50 μg I.V.), CO_2 (0·3 ml. I.C. CO_2-saturated Locke), acetylcholine (ACh, 50 μg I.C.) and to the inhibitor, dopamine (5 μg I.C.), were determined after injections and during infusions of ouabain. Responses to NaCN, CO_2 and ACh increased during the early excitatory period and were greatly reduced during the later period when discharge was decreasing. The effect of dopamine persisted during the excitatory period and was potentiated during the period of reduced discharge.

These results indicate that ouabain can both increase and then reduce the activity of the carotid chemoreceptors. During the excitatory phase chemoreceptor sensitivity to stimulants is increased, whereas during the later phase of decreasing discharge sensitivity to stimulants is reduced and that to inhibitors is increased.

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INHIBITORY ACTIONS OF METHIONINE-ENKEPHALIN AND MORPHINE ON THE CAT CAROTID CHEMORECEPTORS

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1 The effects of intracarotid injections of methionine-enkephalin (Met-enkephalin) and morphine on chemoreceptor activity recorded from the peripheral end of a sectioned carotid sinus nerve have been studied in cats anaesthetized with pentobarbitone.

2 Met-enkephalin caused a rapid, powerful, inhibition of spontaneous chemoreceptor discharge, the intensity and duration of which was dose-dependent.

3 Morphine was a less potent inhibitor of spontaneous chemoreceptor discharge, and the inhibition it evoked was rather variable and tended to be biphasic. Low doses of morphine caused a slight increase in discharge.

4 Naloxone (0.2 mg i.c.) slightly increased spontaneous discharge, greatly reduced the chemo-inhibition caused by morphine, and reduced the inhibitory effect of Met-enkephalin. A higher dose of naloxone (0.8 mg) caused a substantial reduction of the Met-enkephalin effect.

5 Chemo-excitation evoked by intracarotid injections of acetylcholine, CO_2-saturated Locke solution, and sodium cyanide were only slightly and somewhat variably reduced following injections of Met-enkephalin, whereas the inhibitory effect of dopamine was potentiated. Following morphine administration, responses to acetylcholine and sodium cyanide were reduced slightly, whereas those to CO_2 and dopamine were potentiated.

6 Responses to acetylcholine and CO_2 were slightly potentiated during infusion of Met-enkephalin (50 μg/min, i.c.) and the response to sodium cyanide was slightly reduced.

7 It is concluded that naloxone-sensitive opiate receptors are present in the cat carotid body; when activated they cause inhibition of spontaneous chemoreceptor discharge. The physiological role of these receptors and the identity of any endogenous ligand remains to be established.

Introduction

Methionine-enkephalin (Met-enkephalin) is a potent inhibitor of spontaneous chemoreceptor discharge in the cat (McQueen, 1979), and the present neuropharmacological study was undertaken to investigate further this action of Met-enkephalin. It was also considered of interest to determine whether morphine has the same effect as Met-enkephalin on the cat carotid chemoreceptors.

Methods

Experiments were performed on ten cats weighing between 2.1 and 3.5 kg, median weight 2.9 kg. They were anaesthetized with pentobarbitone sodium (42 mg/kg i.p. initially, supplemented by i.v. administration of 10% of the initial dose every 1 to 2 h), artificially ventilated and paralysed by gallamine triethiodide (3 mg/kg i.v.). Full details of the experimental techniques have been given previously (McQueen, 1977; Docherty & McQueen, 1978).

Electrical activity of chemoreceptor units (1 to 5 units) was recorded from filaments of the peripheral end of a sectioned sinus nerve, passed through a pulse height (window) discriminator, and quantified with the aid of a PDP-8 computer. The ganglioglomerular (sympathetic) nerves were cut.

Drugs were dissolved in modified Locke solution (McQueen, 1977). Drug solutions (0.1 ml) were injected into the common carotid artery ipsilateral to the sinus nerve from which activity was being recorded, and washed in with 0.2 ml Locke solution which had been bubbled with 5% CO_2: 95% air in a water bath at 37°C; injections were made over 2 s. The catheter was introduced into the common carotid artery via the lingual artery and advanced until its tip lay about 2 cm caudal to the carotid bifurcation. In

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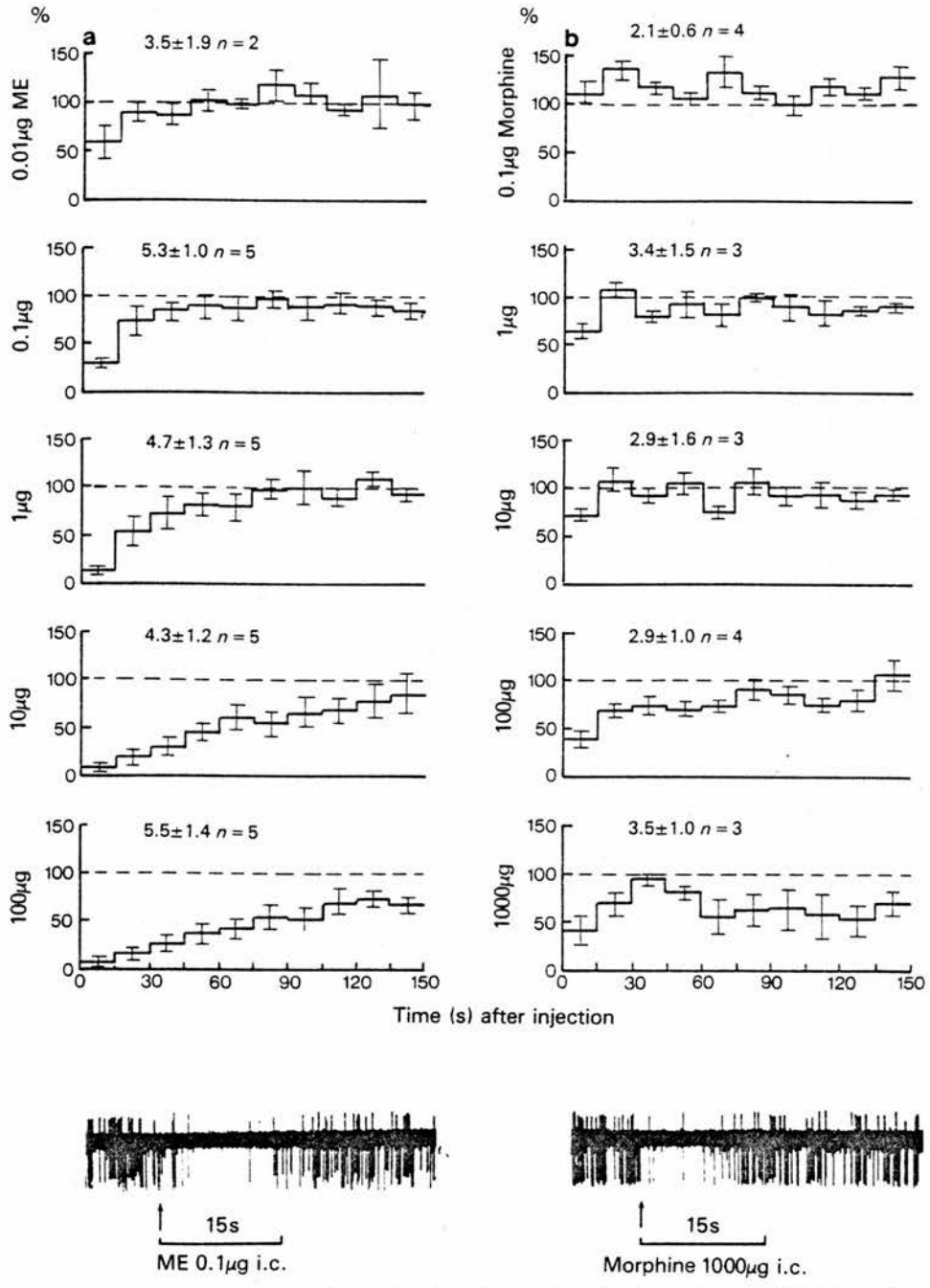

Figure 1 The upper part illustrates the effects of various doses of methionine-enkephalin (ME) (a) and morphine (b) on spontaneous chemoreceptor discharge. Discharge was averaged over 15 s periods following the injection and expressed as a percentage of the averaged discharge in the 15 s pre-injection control period. Data from *n* experiments were pooled and are shown as the mean percentages; vertical lines show s.e. mean. Averaged values (ct/s) ±s.e. mean for the control (100%) periods are given.

The neurograms in the lower part of the figure, taken from one experiment, show the early part of the somewhat similar inhibition of chemoreceptor discharge caused by injecting (arrow) (a) ME 0.1 μg i.c. and (b) morphine 1000 μg i.c.

some experiments a second catheter was positioned in the common carotid artery, this time via the superior thyroid artery, and used for the infusion of drug solutions (0.5 ml/min for 65 s; Braun, Unita).

Drugs used were: morphine sulphate, gallamine triethiodide (May & Baker); sodium cyanide, acetylcholine iodide (B.D.H.); methionine-enkephalin (Uniscience); dopamine hydrochloride (Koch Light) and naloxone hydrochloride (Endo), kindly given to us by Professor W. Feldberg.

Results

Effect of methionine-enkephalin injections on spontaneous chemoreceptor discharge

Met-enkephalin caused a dose-dependent reduction in spontaneous chemoreceptor discharge with a rapid onset, starting within 1 to 2 s of beginning the injection. During the early part of the response there was total inhibition of chemoreceptor discharge, then spontaneous activity returned gradually, reaching control (pre-injection) levels within 30 to 45 s following low doses of Met-enkephalin, but taking up to 5 min to recover after high doses (see Figure 1a). The inhibitory effect was consistent, as can be gauged from the standard errors, and there was no evidence of tachyphylaxis occurring when doses were administered at 7 min intervals. The effect of Met-enkephalin seemed to be potentiated slightly after morphine had been injected. Intravenous injections of Met-enkephalin (10 to 100 μg) also inhibited chemoreceptor discharge, although to a lesser extent and after a longer delay than the same doses by intracarotid injection.

Low doses of Met-enkephalin had little or no effect on blood pressure (BP), whereas higher doses (i.c. or i.v.) caused a fall in BP (see Figure 2).

Effects of morphine injections on spontaneous chemoreceptor discharge

The over-all effect of morphine on spontaneous chemoreceptor discharge was inhibitory, although the lowest dose studied, 0.1 μg, caused a slight increase in discharge (see Figure 1b). Higher doses were associated with an inhibition of discharge which began within 1 to 2 s of starting the injection (Figure 1b) and was dose-related. The initial inhibition lasted for 15 to 45 s after which discharge returned towards pre-injection control levels. There was then a delayed or secondary inhibition of spontaneous discharge, an effect which was most clearly seen after the highest dose of morphine (see Figures 1b, 3a).

Responses to morphine were rather variable, as can be seen from the standard errors, but there was no evidence of tachyphylaxis, and responses to a low dose of morphine injected before and after the highest dose were very similar. The higher doses of morphine caused a fall in BP (see Figure 2).

Effects of naloxone

In an adequately anaesthetized cat, which had received neither Met-enkephalin nor morphine, naloxone (0.2 mg i.c.) caused a slight increase in chemoreceptor discharge and, after a delay of 15 to 45 s, a rise in BP (Figure 2) lasting for at least 30 min. Additional doses of naloxone (0.4, 0.8 and 1.6 mg i.c. at 7 min intervals) had no further effect on chemoreceptor discharge or BP.

When naloxone (0.2 mg i.c.) was injected during experiments in which Met-enkephalin and/or morphine had previously been administered, there was also an increase, albeit somewhat variable, in spontaneous chemoreceptor discharge (Figure 2) and a rise in BP.

Comparison of responses to Met-enkephalin and to morphine obtained before and after injecting naloxone showed that, apart from a slight initial inhibition, the inhibitory action of morphine was virtually abolished, and there was evidence of an over-all increase in discharge. The chemo-inhibitory effect of Met-enkephalin was much reduced (see Figures 2 and 3).

Dose-response data were obtained by expressing the number of impulses in the post-injection period as a percentage of the number of impulses which would have been likely to occur in the same period had the pre-injection control discharge (averaged over 15 to 30 s) continued unaltered, and plotting this value against $\log_{10}$ dose. It can be seen from Figure 3 that naloxone shifts the Met-enkephalin dose-response line to the right, both for 60 s and 150 s post-injection periods. After intracarotid injection of naloxone (0.2 mg), morphine tended to increase discharge over these periods, this effect being inversely related to dose.

A higher dose of naloxone (0.8 mg i.c.) completely abolished the inhibitory action of morphine (1 mg i.c.) and greatly reduced the inhibitory reponse to Met-enkephalin; over-all there was an increase in discharge following lower doses of Met-enkephalin (see Figure 4).

Although naloxone reduced the hypotensive action of Met-enkephalin and morphine, slightly after 0.2 mg i.c. (see Figure 2) and to a greater extent after 0.8 mg i.c., falls in BP were still obtained even when the chemo-inhibitory response had been abolished.

Evoked responses

Injections (i.c.) of acetylcholine (ACh, 50 μg), carbon dioxide-saturated Locke solution (CO_2, 0.3 ml), sodium cyanide (NaCN, 5 μg) and dopamine (5 μg)

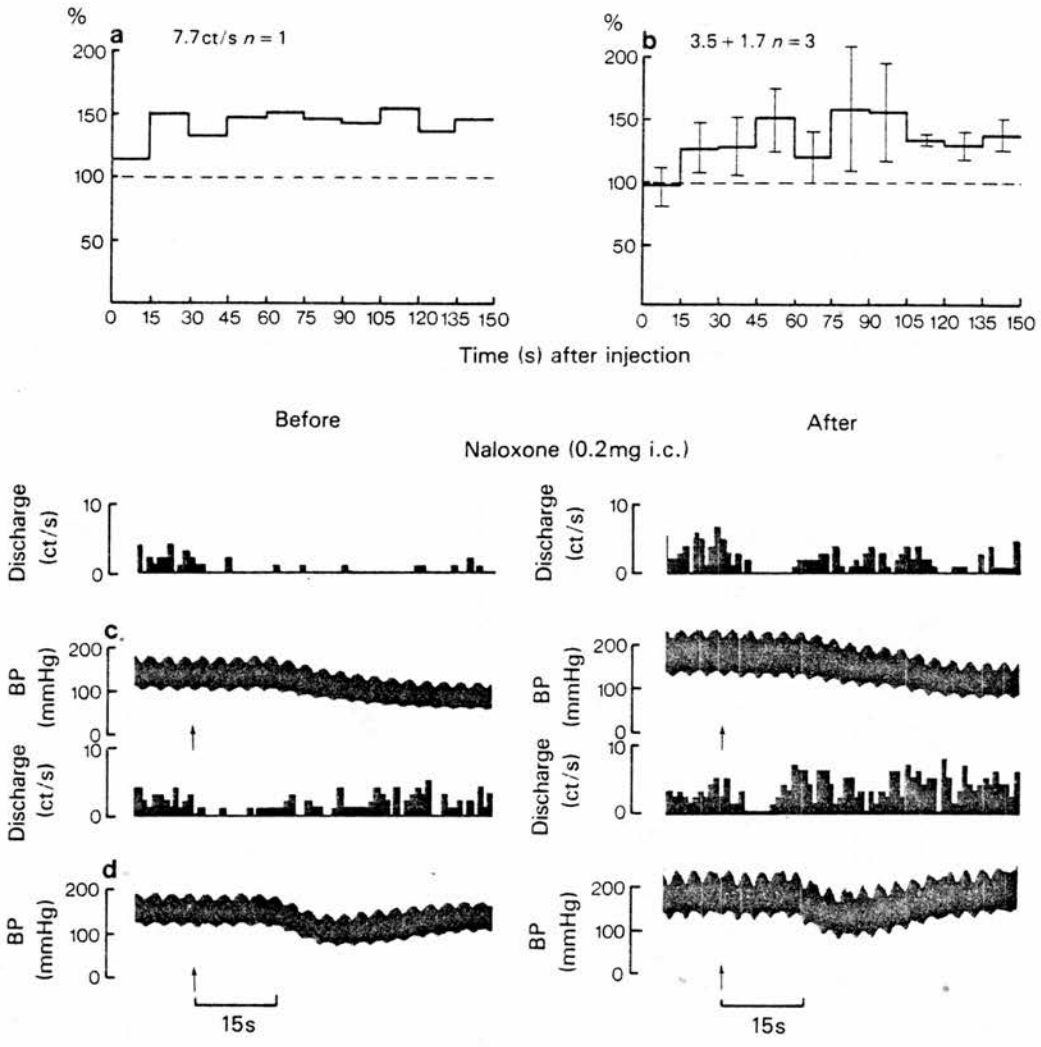

Figure 2 In (a) the effect of naloxone (0.2 mg i.c.) on spontaneous chemoreceptor discharge in an experiment during which no methionine-enkephalin (Met-enkephalin) or morphine had been administered is illustrated; (b) is the averaged spontaneous chemoreceptor discharge from three experiments in which the same dose of naloxone (0.2 mg i.c.) was injected following prior administration of Met-enkephalin and/or morphine. Details as for Figure 1.

Met-enkephalin 100 μg i.c. (c) and morphine 1000 μg i.c. (d) were injected, at the arrows, before and after naloxone (0.2 mg i.c.). Their effects on chemoreceptor discharge and BP are shown, and it can be seen that chemoreceptor inhibition was greatly reduced by naloxone, whereas the hypotension was less affected by this dose of naloxone.

were made before and 5 to 20 min after a series of injections of either Met-enkephalin or morphine. The results obtained (Table 1) showed that the stimulant action of ACh, CO_2, and NaCN were slightly and somewhat variably reduced after Met-enkephalin, whereas the inhibitory effect of dopamine was potentiated. Following morphine administration, responses to ACh and NaCN were reduced slightly, whereas those to CO_2 and dopamine were potentiated.

Responses were also obtained before and during an infusion of Met-enkephalin (50 μg/min i.c.), this dose being sufficient to inhibit spontaneous discharge throughout the infusion period. The effects of ACh and CO_2 were slightly potentiated whereas the response to NaCN was slightly reduced (see Table 1).

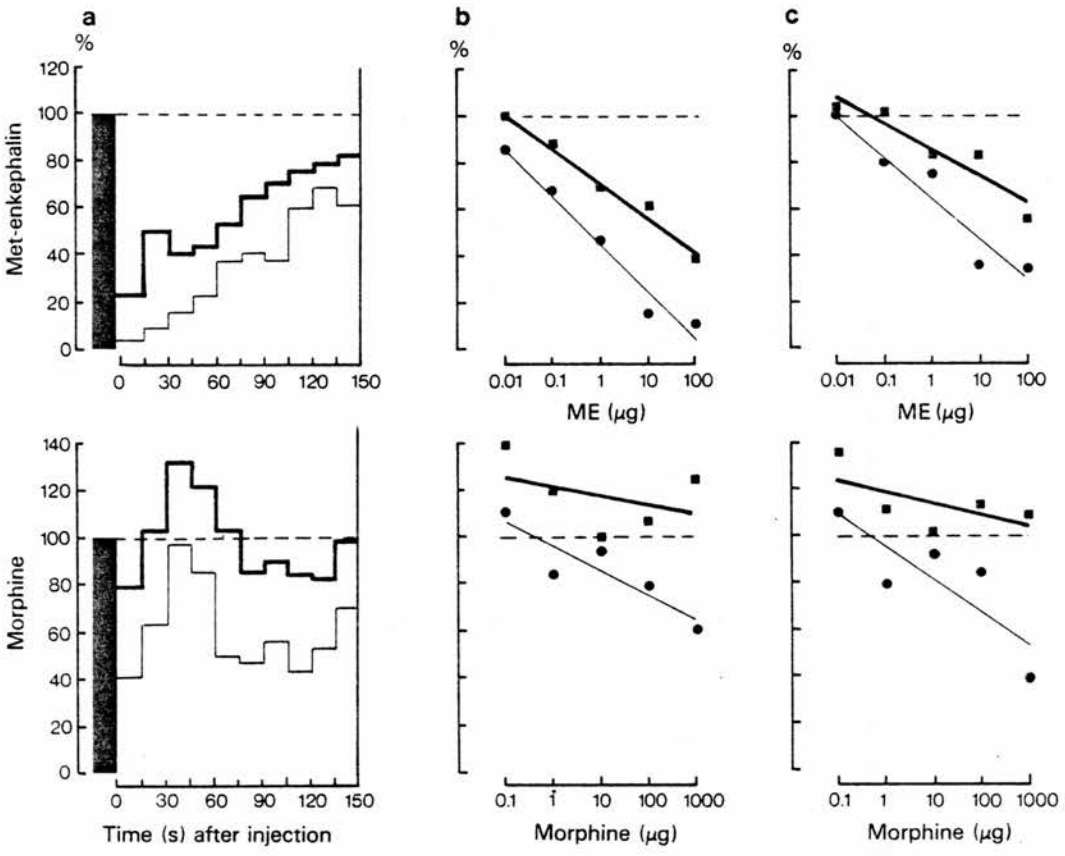

Figure 3 (a) Pooled data showing the effects of methionine-enkephalin (Met-enkephalin) 100 μg (n = 3) and morphine 1000 μg (n = 2) on spontaneous chemoreceptor discharge before (—) and after (—) injecting naloxone (0.2 mg i.c.). Black rectangles represent the control discharge (100%), values for Met-enkephalin being 3.7 ± 1.8 ct/s before and 3.1 ± 0.9 after naloxone, the corresponding values for morphine being 3.7 ± 1.7 and 4.9 ± 2.4 ct/s. See Figure 1 for further details. (b) is a plot of the total discharge over the 60 s post-injection period, expressed as a percentage of the total discharge which would have occurred in the same period if control discharge had continued unaltered (100% = dotted line), against $\log_{10}$ dose of methionine-enkephalin (ME) (pooled data from three experiments) or morphine (data from a single experiment) before (—) and after (—) naloxone (0.2 mg i.c.). Lines were fitted by the method of least squares. (c) is similar to (b), but the plot is of total discharge in the 150 s post-injection period and this includes the delayed inhibition seen with morphine.

Since spontaneous discharge was suppressed it was not possible, under these conditions, to investigate the effect of Met-enkephalin on the inhibitory response evoked by dopamine.

Responses to ACh, NaCN, CO_2 and dopamine were not appreciably affected by naloxone (0.2 mg i.c.).

Discussion

Our results indicate that enkephalin inhibited spontaneous chemoreceptor discharge by acting on naloxone-sensitive receptors in the carotid body. Morphine also acted at these receptors, but it was less potent than Met-enkephalin and caused a more variable inhibition which tended to be biphasic.

Although Met-enkephalin and morphine were able to inhibit spontaneous chemoreceptor discharge, they only slightly reduced excitatory responses evoked by ACh and NaCN, and potentiated dopamine-induced inhibition. These were long-term effects since the responses were not studied until 5 to 20 min after injections of Met-enkephalin or morphine. When responses were studied during an infusion of Met-enkephalin sufficient to reduce spontaneous chemoreceptor discharge substantially, the excitatory effect of NaCN was slightly reduced whereas responses to ACh and CO_2 were potentiated. These single-dose studies are difficult to interpret, particularly since

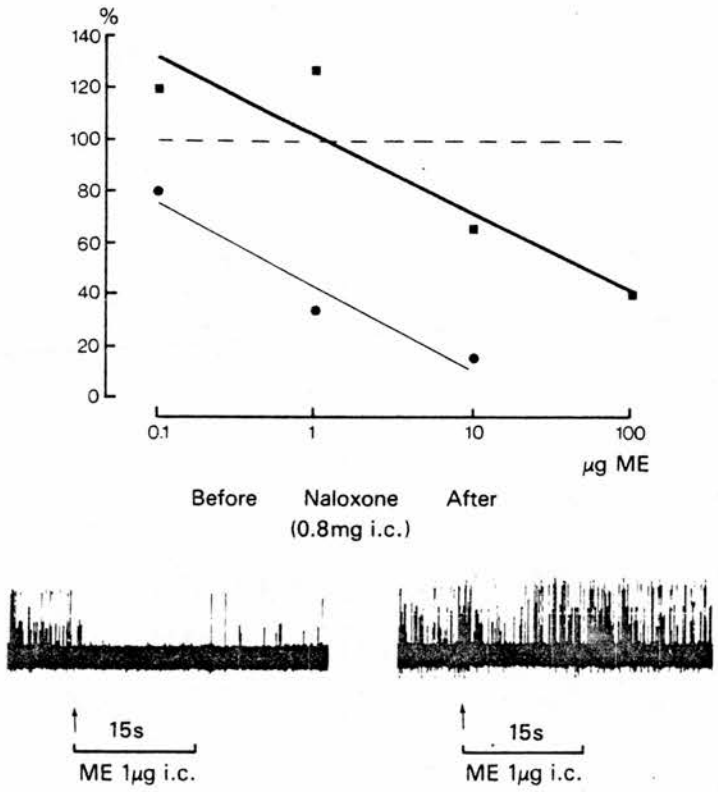

Figure 4 Effects of methionine-enkephalin (ME) on chemoreceptor discharge (plotted as a percentage of the total discharge in the 150 s post-injection period/control discharge × 150 s) before (—) and after (—) injecting naloxone (0.8 mg i.c.). Lines were fitted by the method of least squares. See Figure 3 for further details. Following this dose of naloxone, low doses of Met-enkephalin no longer inhibited chemoreceptor discharge; the tendency was for discharge to be increased. This can be clearly seen in the neurograms which show the effect of Met-enkephalin (1 μg i.c.), injected at the arrow, before and after naloxone (0.8 mg i.c.).

Table 1 Effects of methionine-enkephalin (Met-enkephalin) and morphine on evoked responses

Intracarotid injection of:	*ACh* (50 μg)	*CO_2-Locke* (0.3 ml)	*NaCN* (5 μg)	*Dopamine* (5 μg)	n
A Following morphine injections	83 ± 19	134 ± 11	89 ± 1	159 ± 61	3
B Following Met-enkephalin injections	77 ± 16	72 ± 17	63 ± 10	167 ± 24	5
C During Met-enkephalin infusions (50 μg/min i.c.)	137 ± 23	122 ± 9	84 ± 23	—	2

Pooled data from *n* experiments showing chemoreceptor responses ($\Delta\Sigma x$) (A) following injections of morphine and (B) following injections of Met-enkephalin. The results are expressed as mean percentages ± s.e. mean of the responses to the same doses administered before morphine or Met-enkephalin (i.e. pre-injection response = 100%). Injections were also made 60 s after starting a 65 s infusion of Met-enkephalin and responses evoked compared with pre-infusion values (C).

$\Delta\Sigma x = \Sigma x$ (total spike count during response period, t s) $- (\bar{x}.t)$ where $\bar{x}$ is the average pre-injection (control) discharge in ct/s.

Met-enkephalin may alter blood flow through the carotid body, but the important point is that whereas Met-enkephalin can reduce spontaneous chemoreceptor discharge, it has little effect on responses to intense stimuli of short duration. In the present experiments, the local concentration of Met-enkephalin/morphine may have been high enough to suppress the resting discharge but not that evoked by ACh, NaCN or CO_2.

Injection of the specific opiate antagonist, naloxone (see Sawynok, Pinsky & Labella 1979 for a review), slightly increased chemoreceptor discharge, an effect that was not simply due to reversal of residual Met-enkephalin or morphine chemo-depression because it was also observed when neither substance had been administered. This may mean that there is some tonic inhibition of chemoreceptor discharge by an opioid; alternatively, the effect could be secondary to changes (e.g. in BP) induced by naloxone acting elsewhere. The dose of naloxone used (0.2 mg) is adequate for reversing the fall in BP caused by morphine in cats (Feldberg & Wei, 1977; McQueen, unpublished observations) and for reversing the action of enkephalins in the cat substantia gelatinosa (Duggan, Hall & Headley, 1977). In the present experiments it reduced the chemoreceptor inhibition caused by Met-enkephalin and, to a greater extent, that caused by morphine. It is known that inhibition of neuronal firing caused by morphine is more readily antagonized by naloxone than is that caused by opioid peptides (North, 1979). Increasing the dose of naloxone to 0.8 mg caused an even greater reduction of the Met-enkephalin-induced inhibition of chemoreceptor discharge, and virtually abolished the morphine effect. Higher doses of naloxone could have been studied, but we were concerned that they might have exerted non-specific actions, or reversed the anaesthetic (Fürst, Foldes & Knoll, 1977; Arndt & Freye, 1979; Sawynok *et al.*, 1979). It seems reasonable to conclude that most of the chemoreceptor inhibition results from actions of Met-enkephalin and morphine on naloxone-sensitive receptors in the carotid body.

Low doses of morphine tended to increase spontaneous chemoreceptor discharge, an effect that was potentiated and also obtained with higher doses of morphine, as well as low doses of Met-enkephalin, after naloxone. Landgren, Liljestrand & Zotterman (1952) found that an intracarotid injection of 3 mg morphine hydrochloride in cats caused a moderate increase in small action potentials (probably chemoreceptors) recorded from the sinus nerve, and Eyzaguirre & Zapata (1968) showed that morphine caused a transient increase in discharge recorded from the *in vitro* carotid body preparation. Whether the excitation seen in the present study resulted from an action on naloxone-insensitive opiate receptors, or was caused by morphine/Met-enkephalin influencing substances in the carotid body, as occurs in other tissues (e.g. ACh (Paton, 1957), noradrenaline (Szerb, 1961; Snyder & Childers, 1979), 5-HT, or dopamine (Loh, Brase, Sampath-Khanna, Mar, Way & Li, 1976)), requires further investigation. So does the biphasic nature of the inhibitory response to morphine; could vascular changes be responsible for the transient return of discharge to control levels?

High doses of Met-enkephalin and morphine caused a fall in BP, and it is possible that some of the changes in chemoreceptor discharge might result from changes in blood flow through the carotid body. However, the fact that chemo-inhibitory actions of Met-enkephalin and morphine, (a) started within 1 to 2 s of the injection, (b) occurred following low doses which had no effect on BP and, (c) were greatly reduced by naloxone in doses which only slightly reduced the hypotensive effect, all argue against vascular effects contributing much to the inhibition, at least as far as the early part of the response is concerned. Further information could be obtained by studying the effect of Met-enkephalin on the *in vitro* carotid body preparation (Eyzaguirre & Lewin, 1961), which would eliminate the vascular complications.

The chemo-inhibitory effect of low doses of Met-enkephalin was very similar to that obtained with dopamine, (5 μg i.c.), although it should be noted that Met-enkephalin is 10 to 100 times more potent on a molar basis. However, despite the similarities, it is unlikely that Met-enkephalin acts directly, or, by releasing dopamine within the carotid body, indirectly at a dopamine receptor, because α-flupenthixol blocks the inhibitory action of exogenous dopamine (Docherty & McQueen, 1978) without affecting the inhibitory response to Met-enkephalin (McQueen, 1979).

Substance P is present in the cat carotid body (Cuello & McQueen, 1980) and causes an increase in chemoreceptor discharge on intracarotid injection (McQueen, 1980). It may be that Met-enkephalin is acting to inhibit the release of substance P within the carotid body in the same way as has been shown to occur in the CNS (Jessel & Iversen, 1977), the mechanism probably involving an action of Met-enkephalin on Ca^{2+} channels (Mudge, Leeman & Fischbach, 1979).

It has been suggested that adenosine might be the mediator of the neuro-inhibitory action of opiates (Sawynok & Jhamandas 1976; Stone & Perkins, 1979). Both adenosine (see Ribeiro, 1978) and morphine (Henderson, Hughes & Kosterlitz, 1975) decrease transmitter release in the central and peripheral nervous systems, and adenosine (Ribeiro, Sá-Almeida & Namorado, 1979), morphine (Guerrero-Munoz, Cerreta, Guerrero & Way, 1979), and β-endorphin (Guerrero-Munoz, Guerrero, Way & Li, 1979) decrease the uptake of calcium by synapto-

somes; Ca^{2+} is known to be involved in transmitter release (e.g. Katz & Miledi, 1968). Opioids might depress chemoreceptor activity by inhibiting the entry of Ca^{2+} needed for the release of putative sensory excitatory transmitter(s). Whether adenosine is the mediator of the Met-enkephalin or morphine-induced inhibition, and whether Met-enkephalin interacts with other substances in the carotid body (e.g. substance P, noradrenaline, 5-hydroxytryptamine, dopamine, ACh) requires investigation. Preliminary results (Cuello & McQueen, unpublished observations) suggest that enkephalin-like material is present in the carotid body.

In conclusion, the present results provide pharmacological evidence for the presence of an opiate receptor, or receptors, in the cat carotid body. What type of receptor this is (e.g. Lord, Waterfield, Hughes & Kosterlitz, 1977), where in the carotid body it is located, what the endogenous ligand is and where it originates, and the circumstances under which it is released, all need to be investigated before one can determine whether Met-enkephalin or other opioid peptides have a role as neurotransmitters or neuromodulators (Kosterlitz & Hughes, 1975; Snyder & Childers, 1979) in the cat carotid body chemoreceptors.

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Comparison of the depressant effects of leucine- and methionine-enkephalin on spontaneous chemoreceptor activity in cats

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There is evidence that methionine-enkephalin (met-ENK) and leucine-enkephalin (leu-ENK) are present in cat carotid body type I cells (Lundberg, Hökfelt, Fahrenkrug, Nilsson & Terenius, 1979; Wharton, Polak, Pearse, McGregor, Bryant, Bloom, Emson, Bisgard & Will, 1980). Intra-carotid injection of met-ENK decreases spontaneous chemoreceptor discharge in cats (McQueen, 1979; McQueen & Ribeiro, 1980). The present study was undertaken to investigate the effects of leu-ENK on the carotid chemoreceptors and to compare them with those of met-ENK.

Experiments were performed on cats anaesthetized with pentobarbitone (42 mg/kg i.p., supplemented every 1–2 h). The animals were artificially ventilated with air and paralysed by gallamine (3 mg/kg i.v.). Chemoreceptor activity was recorded from the peripheral end of a sectioned sinus nerve (McQueen, 1977). In the majority of experiments the ganglioglomerular (sympathetic) nerves were cut. Drug solutions were injected into the ipsilateral common carotid artery (i.c.) over a 2 s period.

Leu-ENK and met-ENK both decreased spontaneous chemoreceptor discharge frequency in a dose-dependent manner, being of similar potency (see Figure 1). The intensity and duration of the depression evoked by both peptides were reduced after naloxone (0.2–0.4 mg i.c.). Similar responses were obtained in all the experiments, regardless of whether or not the sympathetic nerve supply to the carotid body had been cut.

These results suggest that leu-ENK and met-ENK depress spontaneous chemoreceptor discharge by acting on opiate receptors in the carotid body. Since the enkephalins have potent effects on chemoreceptor discharge and are present in the carotid body, they may function there as neurotransmitters or neuromodulators. Further studies are needed to establish their physiological roles in the carotid body and to identify the opiate receptor(s) (Hughes, Kosterlitz, McKnight, Sosa, Lord & Waterfield, 1978).

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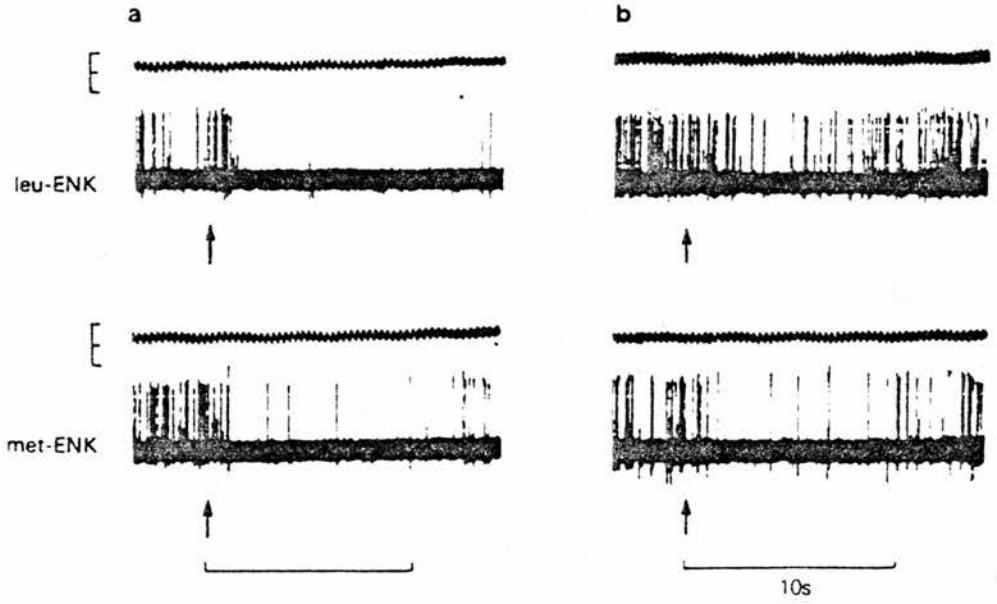

Figure 1 Effects of i.c. injections (arrows) of leu-ENK (10 μg) and met-ENK (10 μg) on spontaneous chemoreceptor activity before (*a*) and after (*b*) naloxone (0.4 mg i.c.). leu-ENK was injected 5 min after naloxone and met-ENK 10 min later. The upper part of each panel shows the femoral arterial B.P. (calibration 0, 100, 200 mmHg), and the lower part the neurogram.

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EFFECTS OF β-ENDORPHIN, VASOACTIVE INTESTINAL POLYPEPTIDE AND CHOLECYSTOKININ OCTAPEPTIDE ON CAT CAROTID CHEMORECEPTOR ACTIVITY

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SUMMARY

The effects of β-endorphin, vasoactive intestinal polypeptide (VIP) and cholecystokinin octapeptide (CCK-8) on carotid chemoreceptor activity have been investigated in cats anaesthetized with pentobarbitone. Spontaneous chemoreceptor discharge was decreased by intracarotid injection of β-endorphin and by low doses of VIP, whereas it was increased by CCK-8 and higher doses of VIP, these effects being relatively long-lasting and often associated with changes in systemic blood pressure. The chemoexcitation evoked by acetylcholine and sodium cyanide was reduced during intracarotid infusion of any of the three peptides studied, and that caused by CO_2-saturated Locke solution was reduced by β-endorphin, largely unaltered by VIP and variably affected by CCK-8. The inhibitory effect of β-endorphin was greatly reduced by naloxone, implying that it probably involved actions at naloxone-sensitive opiate receptors in the carotid body. Substance P was unable to overcome the chemoinhibitory effect of methionine enkephalin. Possible functions of polypeptides in the carotid body are discussed.

INTRODUCTION

The ability of some polypeptides to modify spontaneous chemoreceptor discharge when injected close-arterial to the cat carotid body has been described (McQueen, 1979, 1980). Since then evidence has been presented which shows that substance P (SP), the enkephalins and vasoactive intestinal polypeptide (VIP), or closely related immunoreactive substances, are present in the cat carotid body (Lundberg, Hökfelt, Fahrenkrug, Nilsson & Terenius, 1979; Cuello & McQueen, 1980; Wharton, Polak, Pearse, McGregor, Bryant, Bloom, Emson, Bisgard & Will, 1980).

The present neuropharmacological study was undertaken to investigate further the actions of various polypeptides, including those identified as being present in the carotid body, on the cat carotid chemoreceptors with a view to obtaining some insight into the physiological role of these substances in the carotid body.

METHODS

Experiments were performed on nine cats weighing between 3·0 and 4·4 kg, median weight 3·5 kg. They were anaesthetized with pentobarbitone sodium (42 mg . kg^{-1} I.P.), supplemented as required during the experiments, artificially ventilated with air and paralysed with gallamine (3 mg . kg^{-1} I.V.). Full details for most of the experiment procedures have been given previously (McQueen, 1977; Docherty & McQueen, 1978) and only a brief description follows.

The lingual and superior thyroid arteries ipsilateral to the sinus nerve from which recordings were

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obtained were both cannulated, the catheter tips being positioned in the common carotid artery; blood pressure was recorded from a femoral artery. Electrical activity of chemoreceptor units (1–5 units) was recorded from filaments of the peripheral end of a sectional sinus nerve, stored on FM tape, passed through a pulse height (window) discriminator and quantified with the aid of a PDP-8 computer. The ganglioglomerular (sympathetic) nerves were cut.

Drugs were dissolved in either modified Locke solution (see McQueen, 1977) or 0·9% w/v aqueous sodium chloride solution, except β-endorphin which was initially dissolved in 0·5% aqueous bovine serum albumin solution, then diluted with saline. *Injections* were made in a volume of 0·1 ml into the lingual catheter and washed in with 0·2 ml Locke solution which had been bubbled with 5% CO_2:95% air in a water bath at 37 °C; they were made over a 2 s period. *Infusions* were made into the common carotid artery via the thyroid catheter at a rate of 0·1 ml.min^{-1}, using a Unita pump (Braun), and lasted for 5–10 min; the catheter dead-space was 0·2 ml.

Drugs used were: pentobarbitone sodium, gallamine triethiodide (May & Baker), acetylcholine iodide, sodium cyanide (B.D.H.), dopamine hydrochloride (Koch Light), substance P, methionine enkephalin formate (Sigma), cholecystokinin octapeptide non-sulphated (Peninsula Labs), naloxone hydrochloride (Endo), human β-endorphin (Aalton Bio Reagents, Dublin), porcine β-endorphin (kindly given to us by Dr D. Smyth, National Institute for Medical Research, London) and vasoactive intestinal polypeptide (kindly given to us by Dr S. I. Said, University of Texas Health Science Center, Dallas, U.S.A.).

RESULTS

β-endorphin

In three experiments the effects of β-endorphin on chemoreceptor activity were examined. Since there was no appreciable difference between the actions of human and porcine β-endorphin, results have been expressed simply as responses to β-endorphin.

Injections of β-endorphin. Injections of β-endorphin (0·1–50 μg I.C.) caused a decrease in spontaneous chemoreceptor discharge (see Fig. 1 *B* and *C*). The response lasted for about 3 min following the highest dose investigated (50 μg) and was associated with a very slight fall in systemic blood pressure (Fig. 1 *A*). Discharge was averaged over the 60 s period immediately following β-endorphin injection and expressed as a percentage of the averaged pre-injection (control) discharge (Fig. 1 *D*). The results showed that the decrease in discharge was dose-dependent and, from consideration of discharge during the second minute following the injection (Fig. 1 *E*), fairly long-lasting. The reason for the somewhat variable nature of the response, reflected in the scatter of points about the lines, was that responses to β-endorphin were often bi- or triphasic; there was an initial decrease followed by a return to control level, or to discharge frequencies greater than control, then a delayed decrease in spontaneous discharge (see Fig. 2 *A*). The overall effect was similar to that evoked by morphine (McQueen & Ribeiro, 1980).

Following administration of the opiate antagonist naloxone (0·4 mg I.C.) β-endorphin caused only a slight decrease in spontaneous chemoreceptor discharge, the main effect being a rather variable increase in discharge (Fig. 2 *B*) which was not abolished by additional doses of naloxone.

Infusions of β-endorphin. Infusion of β-endorphin at a rate of 5 μg/min for 5–10 min resulted in very slight changes in spontaneous chemoreceptor discharge and systemic blood pressure (Fig. 3 *A*). Blood gas tensions and pH were unaffected by the infusion.

Evoked responses during infusion of β-endorphin. The increases in chemoreceptor discharge evoked by ACh, CO_2 and NaCN, together with the decrease caused by dopamine, were determined before, during, and after an infusion of β-endorphin at a rate of 5 μg/min. The

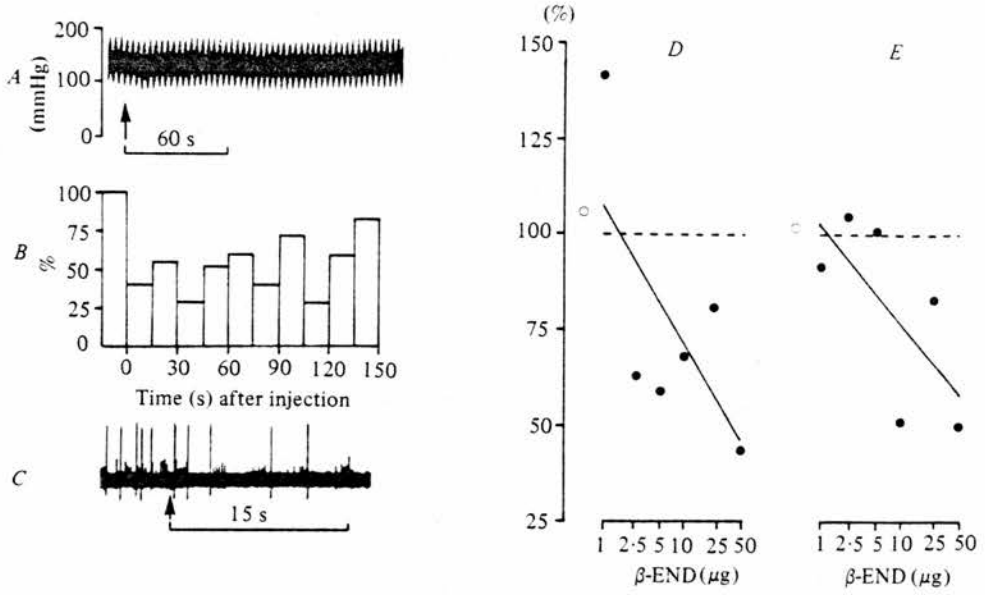

Fig. 1. The left-hand side of the figure shows the effect of injecting (arrow) β-endorphin (50 μg I.C.) on: *A*, systemic arterial blood pressure; *B*, spontaneous chemoreceptor discharge averaged over a 15 s periods and expressed as a percentage (control discharge, 1·5 ct/s = 100%); *C*, the single chemoreceptor unit from which the data in *B*, *D* and *E* were obtained. The right-hand side of the figure shows the discharge averaged over the first (*D*) or second (*E*) minute following β-endorphin injection. Discharge was expressed as a percentage of the pre-injection frequency and plotted against dose of β-endorphin ($\log_{10}$ scale), straight lines being fitted to the responses (●) by the method of least squares. ○, illustrate the effect of injecting the drug vehicle (0·5% bovine serum albumin): the pre-injection (control) discharge frequency (100%) was determined by averaging spontaneous discharge during the 30–60 s immediately preceding each injection, and is represented by the dotted line.

results obtained are summarized in Fig. 4 and it was found that responses to the stimulants were reduced by β-endorphin in the concentration studied, whereas the inhibitory effect of dopamine was potentiated. The influence of β-endorphin on responses evoked by dopamine and, to a lesser extent, NaCN, was still evident 15 min after the infusion had finished.

In a separate experiment the influence of β-endorphin (5 μg/min) on the inhibition evoked by an injection of methionine enkephalin (10 μg I.C.) was studied. The effect of methionine enkephalin was found to be very slightly enhanced. (Discharge, averaged over 150 s after the enkephalin injection and expressed as a percentage of the pre-injection discharge frequency (100%) was 71% before the infusion, 64% during the infusion, and 73% 15 min after the infusion of β-endorphin.)

Vasoactive Intestinal Polypeptide (VIP)

In two experiments the effects of VIP were studied. Only a limited amount of VIP was available, but the results obtained were quite clear.

Injections of VIP. VIP injections (0·05–6·3 μg I.C.) produced the type of effects seen in Fig. 5. Low doses caused a decrease in spontaneous chemoreceptor discharge whereas higher doses increased discharge. The effects were fairly long-lasting, being evident in the second minute following the injection (see Fig. 5*D*). There was a good correlation between dose and response, and higher doses caused a fall in B.P. followed by a rise (Fig. 5*A*).

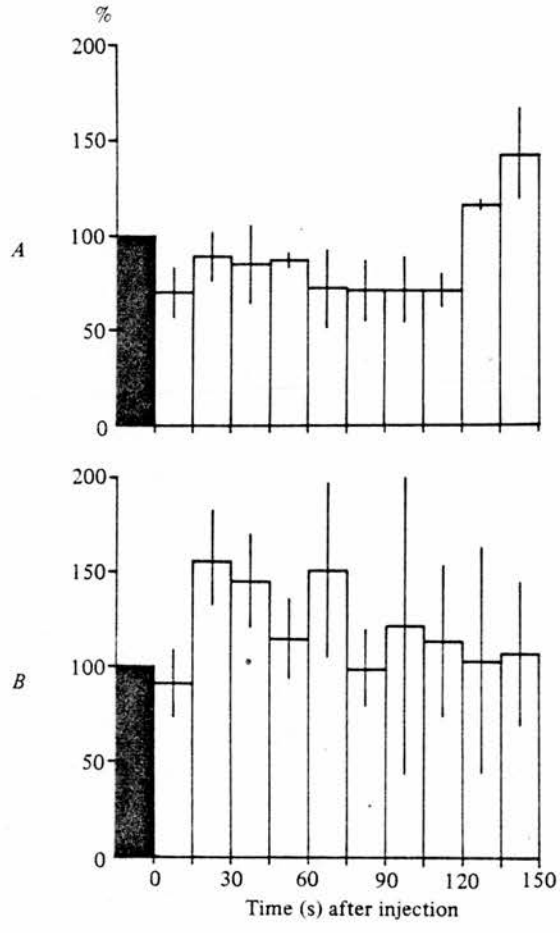

Fig. 2. Chemoreceptor discharge following injection of β-endorphin 10 μg I.C., *A*, before and *B*, 5 min after naloxone (0·4 mg I.C.). Pooled data from three experiments, discharge expressed as a percentage of the pre-injection frequencies and shown as the average $\pm$ S.E. of mean in 15 s intervals during the 150 s post-injection period. The averaged control values were 4·8 $\pm$ 2·0 ct/s before and 2·7 $\pm$ 1·6 ct/s after naloxone.

Infusions of VIP. Infusion of VIP at a rate of 0·5 μg/min for 5–10 min caused a sustained increase in discharge and a fall in blood pressure (see Fig. 3 *B*). The chemoreceptor response shown in Fig. 3 *B*, the biggest observed with the dose of VIP studied, was accompanied by a slight fall in B.P. The control B.P. was rather low, and to preclude the possibility that the increase in chemoreceptor discharge was entirely secondary to the systemic vascular effects of VIP, the infusion was repeated later in the same experiment when mean systemic B.P. was over 100 mmHg and dextran was infused to prevent the fall in B.P. Under these conditions discharge still increased, although the increase was a little less than that obtained at the lower pressure. Measurement of arterial blood gases and pH (see Fig. 3, legend) showed that the increase in chemoreceptor discharge was not secondary to changes in arterial blood gas tensions.

Evoked responses during infusion of VIP. Responses to ACh, CO_2 and NaCN were determined before, during and after infusions of VIP at a rate of 0·5 μg/min. The results

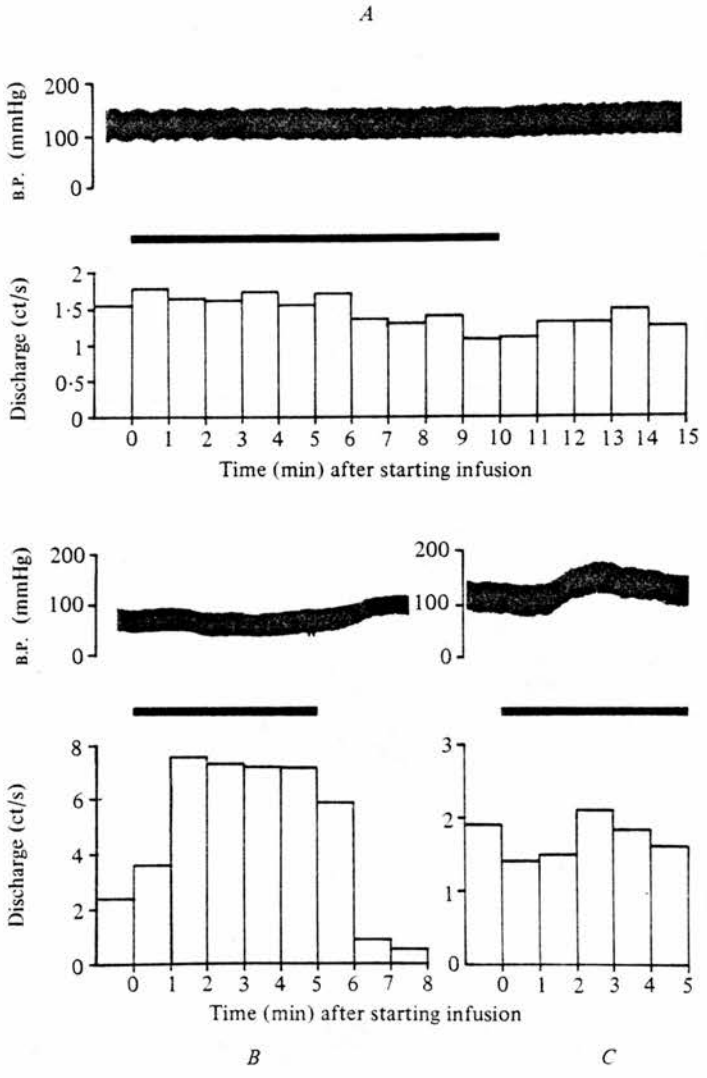

Fig. 3. Effects on spontaneous chemoreceptor discharge of infusing, during the periods represented by the horizontal bars, *A*, β-endorphin 5 μg/min I.C.; *B*, VIP 0·5 μg/min I.C.; *C*, CCK-8 10 μg/min I.C. Discharge (ct/s) was averaged over 60 s intervals, commencing 60 s before starting an infusion, and plotted on the same time scale as the B.P. trace. Just before the end of the VIP infusion in *B*, an arterial blood sample was taken and gave values of pH 7·31, $P_{a.CO_2}$ 107 mmHg, $P_{a.CO_2}$ 34 mmHg compared with pre-infusion sample values of pH 7·28, $P_{a.CO_2}$ 105 mmHg, $P_{a.CO_2}$ 36 mmHg. These results were obtained from three separate experiments, and it should be noted that the catheters were primed with polypeptide solution (i.e. there was no dead-space to be cleared after starting an infusion).

obtained from two experiments were pooled and are summarized in Fig. 4. It was found that responses to the stimulants were reduced by VIP, whereas the inhibitory effect of dopamine was only slightly reduced during the infusion, although there was a further reduction after infusion. Responses to ACh and CO_2 recovered within 15 min, whereas the NaCN and dopamine responses did not.

In one experiment the influence of a VIP infusion (0·5 μg/min) on the inhibition evoked by methionine enkephalin (10 μg I.C.) was studied. Before the VIP infusion chemoreceptor

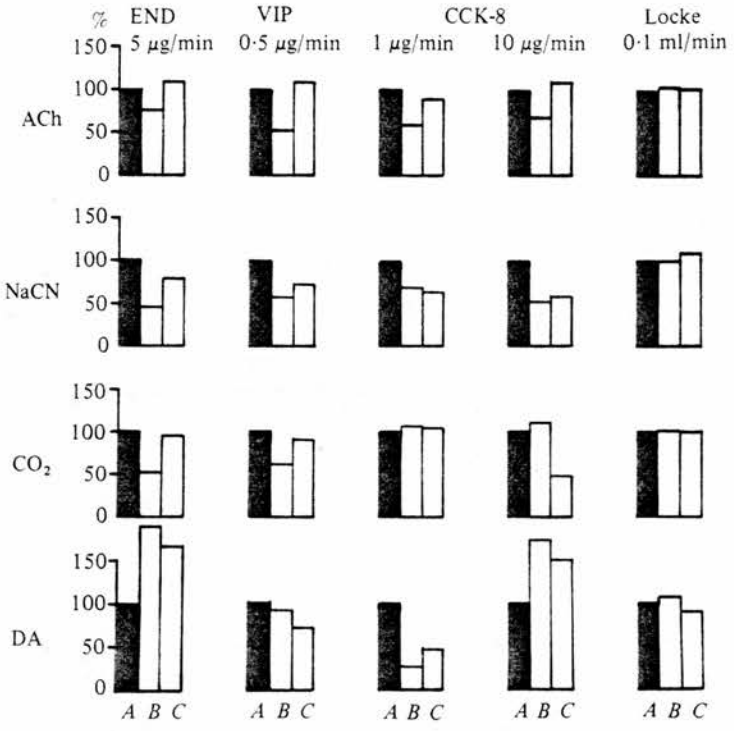

Fig. 4. Responses ($\Delta\Sigma x$) to ACh (50 μg I.C.), NaCN (2·5 μg I.C.), CO_2 (0·3 ml CO_2-saturated Locke solution) and dopamine (DA, 5 μg I.C.) were obtained; *A*, before a 10 min infusion of polypeptide into the second carotid catheter; *B*, during the infusion; *C*, 5–15 min after finishing the infusion, and are shown as percentages (pre-infusion response = 100%). In the case of β-endorphin and CCK-8 data were from single experiments; for VIP and Locke solution the data shown are averages from two experiments. $\Delta\Sigma x = \Sigma x - \bar{x} \cdot t$, where Σx is the number of action potentials counted during the response of duration t s, a 'response' being defined as lasting from the first substantial change from background discharge frequency ($\bar{x}$ ct/s) until return to background level.

discharge averaged over the immediate 60 s post-injection period was reduced to 27% of the pre-injection control frequency, and over the second minute it was reduced to 89% of control. Corresponding values during VIP infusion were 46% in the first minute and 100% in the second, meaning that the inhibition caused by methionine enkephalin was reduced during the VIP infusion.

Cholecystokinin octapeptide (CCK-8)

In two experiments the effects of CCK-8 on chemoreceptor activity were examined. Similar effects were obtained in both experiments.

Injections of CCK-8. Injections of CCK-8 (0·1–100 μg I.C.) caused a somewhat variable short-lasting biphasic effect on discharge, generally an initial decrease followed by an increase (see Fig. 6). Higher doses caused a rise in B.P. The quantitative evidence shown in Fig. 6*C* and *D* confirms that responses were somewhat variable and short-lived.

Infusions of CCK-8. In one experiment CCK-8 was infused at a rate of 1 μg/min, in the other the rate was 10 μg/min. There was little effect on spontaneous discharge with either dose (Fig. 3*C*); arterial blood pressure rose.

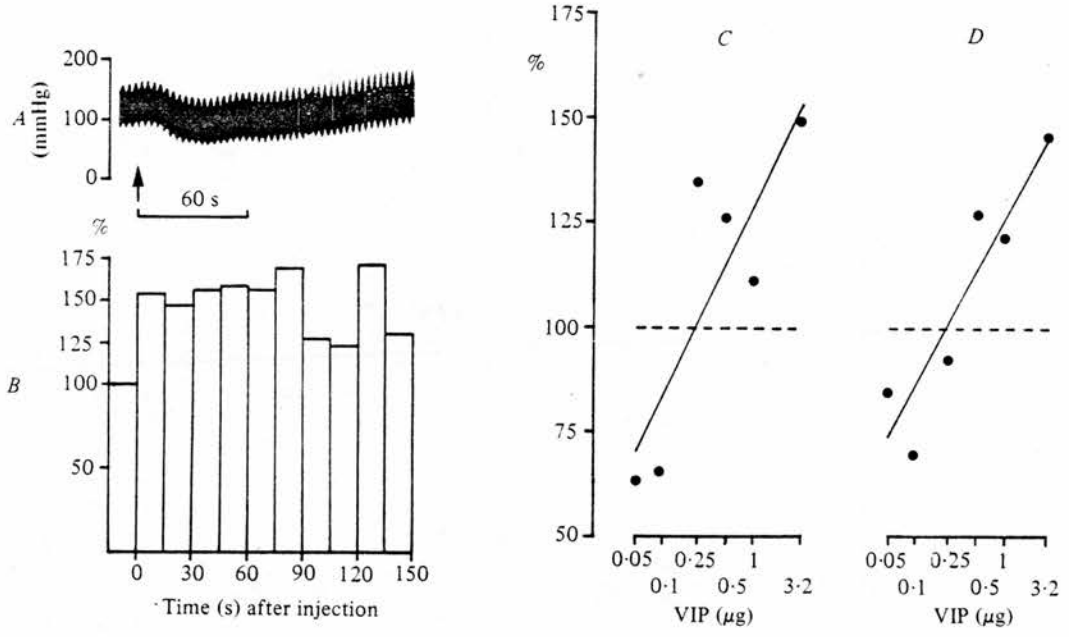

Fig. 5. The left-hand side of the figure shows the effect of injecting VIP (3·2 μg I.C.) on: *A*, systemic arterial blood pressure; *B*, spontaneous chemoreceptor discharge, control frequency (100%) being 5·6 ct/s. The right-hand side of the figure shows data obtained from the same experiment and illustrates discharge averaged over the first (*C*) or second (*D*) minute following VIP injection. Figure details as for Fig. 1.

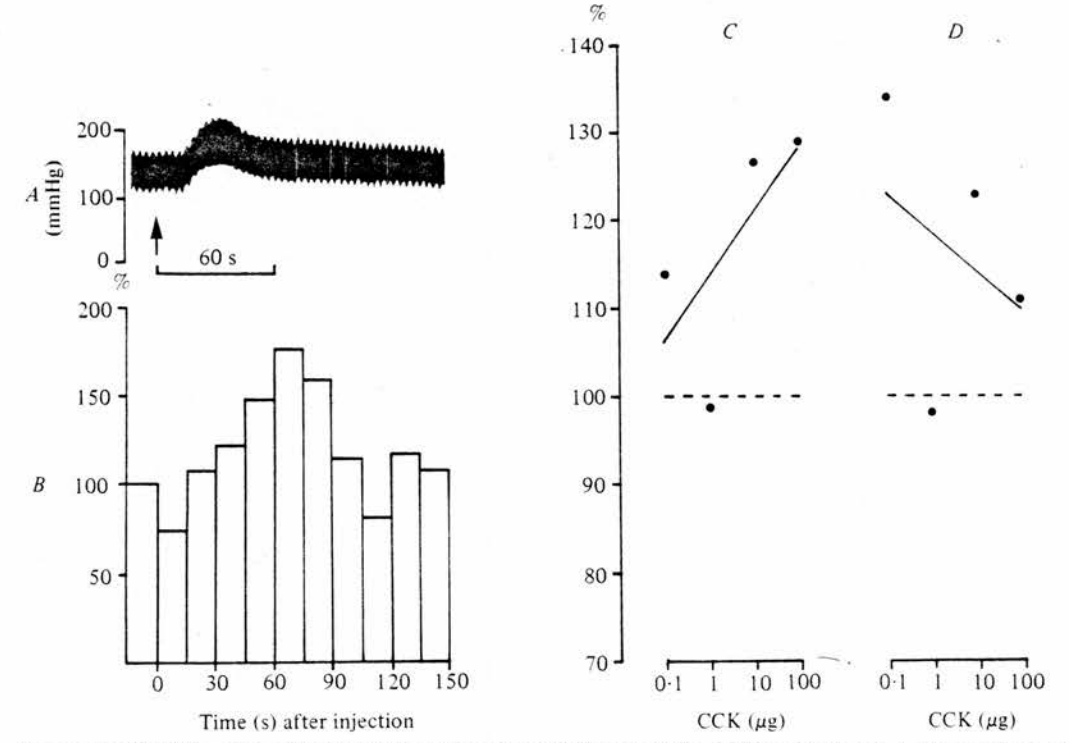

Fig. 6. The left-hand side of the figure shows the effect of injecting CCK-8 (100 μg I.C.) on: *A*, systemic arterial blood pressure; *B*, spontaneous chemoreceptor discharge, control frequency (100%) being 1·9 ct/s. The right-hand side of the figure shows pooled data from two experiments, illustrating discharge averaged over the first (*C*) or second (*D*) minute following CCK-8 injection. Figure details as for Fig. 1.

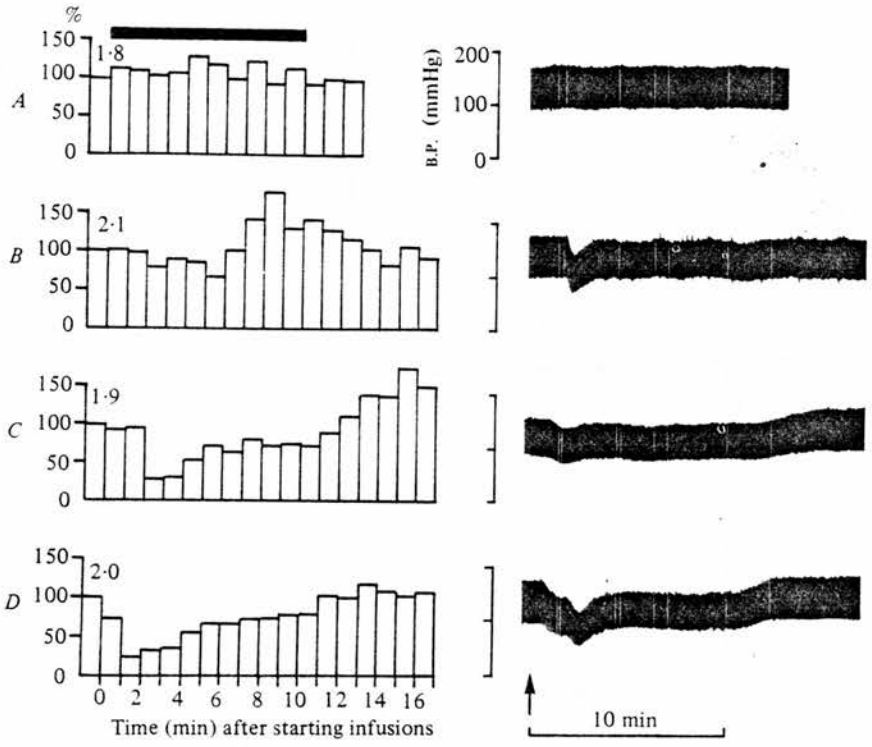

Fig. 7. The left-hand side of the figure shows chemoreceptor discharge (control (100%) values given in ct/s) averaged over 60 s periods after starting intracarotid infusions (horizontal bar) of: *A*, Locke solution via lingual and thyroid catheters; *B*, SP 25 μg/min via the lingual, Locke solution via the thyroid catheter; *C*, methionine enkephalin 10 μg/min via the thyroid, Locke solution via the lingual; *D*, methionine enkephalin 10 μg/min via the thyroid, SP 25 μg/min via the lingual. The accompanying blood pressure records are shown on the right-hand side of the figure, the time scale being common to both sides. The catheter dead-space needed to be cleared before the peptides arrived in the carotid artery, a process which took 1–2 min after starting the infusion, except in *D* where the thyroid catheter was fully primed with methionine enkephalin so that the action of this peptide had begun before SP reached the carotid artery.

Evoked responses during infusion of CCK-8. Responses to ACh, NaCN and DA were reduced during infusion of CCK-8 at a rate of 1 μg/min. The response to CO_2 was unaffected (Fig. 4). Although the ACh effect returned to pre-infusion levels within 15 min of finishing the infusion, responses to NaCN and dopamine remained depressed. In a second experiment, during which CCK-8 was infused at 10 μg/min, responses were somewhat similar except that a sustained potentiation of the dopamine-induced chemoinhibition was observed and there was a decreased response to CO_2 in the post-infusion period, although not during the infusion (Fig. 4).

Substance P and methionine enkephalin

Previous experiments had established that both these polypeptides can influence chemoreceptor discharge (McQueen, 1979; McQueen & Ribeiro, 1980) and the present experiment was undertaken to determine whether any interaction occurs on simultaneous infusion of both peptides close-arterial to the carotid body. The results obtained are shown in Fig. 7. Locke solution caused a very slight increase in spontaneous chemoreceptor discharge, SP had a biphasic action (decrease followed by increase) and methionine enkephalin reduced

discharge, an effect which was most pronounced in the early part of the response; when the methionine enkephalin infusion was stopped discharge increased above pre-infusion control levels. The delayed increase in discharge seen with SP was accompanied by a rise in end-tidal CO_2; there was no change in end-tidal CO_2 during or after methionine enkephalin infusion.

SP and methionine enkephalin were infused concurrently in equimolar concentrations, it being so arranged that methionine enkephalin began to act before SP reached the carotid artery (see Fig. 7). The pattern of response was very similar to that obtained with methionine enkephalin alone, except that no post-infusion overshoot occurred. There was a rise in end-tidal CO_2 towards the end of the infusion. Both peptides caused hypotension, and when given together the overall effect on B.P. was approximately additive.

DISCUSSION

The present study showed that the polypeptides investigated can modify carotid chemoreceptor activity in the cat. Spontaneous chemoreceptor discharge was decreased by intracarotid injection of β-endorphin and by low doses of VIP, whereas it was increased by CCK-8 and higher doses of VIP. The chemo-excitatory effect of VIP has been reported by Fitzgerald, Raff, Garger, Fechter, Anand & Said (1979). The effects of these peptides on chemoreceptor discharge may not seem very impressive when compared with those of ACh, NaCN, CO_2 or dopamine, but it has to be remembered that the polypeptides have a relatively high molecular mass so that, for example, whereas the excitatory response to ACh was elicited by 180 nmol (50 μg), that to VIP involved only 1 nmol (3·2 μg). Furthermore, although the peptide effects were generally less intense they tended to be longer lasting than those associated with classical neurotransmitters. The prolonged action may be related to various factors, such as molecular size, tissue penetration, dissociation from receptors, 'activation of 'second messengers', or to peptide inactivation, and needs to be further investigated.

Responses evoked by ACh, NaCN, CO_2 and dopamine during polypeptide infusion, when peptide levels in the carotid body were assumed to be relatively steady, showed that the excitatory action of ACh and NaCN was reduced by all three peptides studied, and that caused by CO_2 was reduced by both β-endorphin and VIP, but unaffected by CCK-8. Dopamine-induced chemoinhibition was potentiated by β-endorphin, largely unaltered by VIP and reduced by low concentrations of CCK-8 but potentiated by higher concentrations. Lack of sufficient peptide precluded full dose response studies (e.g. McQueen, 1977), but although the results obtained with single submaximal doses may be less reliable, they do indicate that polypeptides can influence evoked response when infused in fairly low concentrations. It is particularly intriguing that polypeptides are sometimes stored with putative neurotransmitters (see Pearse, 1969), for example VIP with ACh (Lundberg, Hökfelt, Schultzberg, Uvnäs-Wallensten, Köhler & Said, 1979), enkephalin with noradrenaline (Schultzberg, Lundberg, Hökfelt, Terenius, Brandt, Elde & Goldstein, 1978), SP with 5-hydroxytryptamine (Hökfelt, Ljungdahl, Steinbusch, Verhofstad, Nilsson, Brodin, Pernow & Goldstein, 1978) and CCK-8 with dopamine (Hökfelt, Johansson, Ljungdahl, Lundberg & Schultzberg, 1980). All these putative neurotransmitters are present in the carotid body (see Biscoe, 1972) and so, it appears, are several of the polypeptides. It will be most interesting to determine whether similar dual localization of peptide and amine occurs in the carotid body and whether the distribution of peptides and peptides/amines within the carotid body is homogeneous, or whether different regions or nerve fibres (A

and C, afferent, efferent) have different representation. It may transpire that all the different substances in the carotid body, although activating different receptors, eventually influence a common mechanism (e.g. adenylate cyclase) within the carotid body.

The action of β-endorphin on the chemoreceptors was similar to that of morphine (McQueen & Ribeiro, 1980), there being a tendency for inhibitory responses to be biphasic, and greatly reduced by naloxone (0·4 mg I.C.). Following naloxone an overall increase in discharge occurs (Fig. 2). These similarities might mean that both substances affect the same receptors, with β-endorphin being about twenty times more potent than morphine on a molar basis. The enkephalins, in contrast, cause a much greater chemoinhibition which is not so readily prevented by naloxone, and also have greater hypotensive effects (McQueen & Ribeiro, 1980, 1981). Thus, the chemoinhibition caused by β-endorphin appears to result from actions on naloxone-sensitive opiate receptors, whereas the slight chemoexcitation seen after naloxone does not. The latter effect may result from actions at opiate receptors which are insensitive to naloxone in the doses used, or to direct or indirect actions on non-opiate receptors.

The polypeptides influenced arterial blood pressure and this confirmed that, in the doses studied, they were biologically active. The possibility that the effect of polypeptides on chemoreceptor activity was secondary to vascular changes either within the carotid body, or systemically, needs to be considered. The rapid onset of the peptide effect (within 1–2 s of injection), the fact that effects on chemoreceptor discharge occurred before changes in blood pressure were seen and persisted when such changes were prevented, and the knowledge that chemoreceptor discharge appears to be largely independent of blood pressure over the physiological range (Hornbein, Griffo & Roos, 1961; Biscoe, Purves & Sampson, 1970; Acker, Keller, Lübbers, Bingman, Schulze & Caspers, 1973) and probably carotid body blood flow (Acker & Lübbers, 1977) lead us to consider it unlikely that vascular effects were responsible for the greater part of the responses observed.

The fact that most of the peptides affect the vasculature could be taken to imply that the role of endogenous carotid body peptides is to modify blood flow. However, it is not known whether endogenous polypeptides do in fact modify carotid body blood flow when released, and many established neurotransmitters, such as ACh, noradrenaline and dopamine, have marked vascular effects when injected. This, albeit together with other evidence, has not prevented these latter substances, when present in the carotid body (e.g. see Biscoe, 1972), from being considered as putative chemoreceptor neurotransmitters, so by the same token the vascular effects caused by exogenous polypeptides should not exclude them from consideration as putative neurotransmitters when found to be present in the carotid body.

During the present study we investigated whether any interaction occurred in the carotid body between SP and methionine enkephalin. These two peptides, or closely related material, are present in the cat carotid body (see Introduction) and SP has been claimed to be a neurotransmitter at the central nerve terminals of baro- and chemoreceptor afferent fibres of cats (Gillis, Helke, Hamilton, Norman & Jacobowitz, 1980) and rats (Helke, O'Donohue & Jacobowitz, 1980; Jacobowitz & Helke, 1980). It is also known that methionine enkephalin can inhibit the release of SP from cultured sensory neurones (Mudge, Leeman & Fishbach, 1979) and probably in the c.n.s. (Jessel & Iversen, 1977). Our results showed that SP was unable to overcome the chemoinhibitory effect of methionine enkephalin, something it might have been expected to do if release of endogenous SP is crucial for chemoexcitation, and can be prevented by methionine enkephalin. However, much may depend on the type of fibre being recorded and the background level of activity;

SP is particularly associated with C fibres (Hökfelt, Johansson, Kellerth, Ljungdahl, Nilsson, Nygårds & Pernow, 1977) and it could be that A fibres were being recorded which are, perhaps, affected more by methionine enkephalin than by SP. In the absence of conduction velocity measurements this must remain speculation, and also we don't know whether the concentration of injected SP in the carotid body was physiological. As far as the other polypeptides were concerned, the inhibition evoked by methionine enkephalin was slightly potentiated by β-endorphin but reduced by VIP.

Evidence from other parts of the nervous system suggests that some polypeptides may function as neurotransmitters (see Hökfelt *et al.* 1980), but the question of what physiological role the polypeptides play in the nervous system is still very much a matter for debate (see Bishop & Polak, 1978; Guillemin, 1978; Hökfelt *et al.* 1980; Snyder, 1980). As far as the carotid body is concerned, various exogenous polypeptides affect chemoreceptor activity and several of these are known to be present in the structure. However, on its own this information does not enable us to reach a conclusion regarding the role of the peptides in the carotid body. They may function as neurotransmitters, neuromodulators, co-transmitters affecting receptor sensitivity, neurohormones, trophic factors, or as agents modifying the vasculature, and might be influenced by each other, by carotid body amines, by circulating hormones (e.g. ACTH, β-endorphin) or by other substances.

In conclusion, further studies are needed in order to determine the role of polypeptides in carotid body chemoreception, and these will involve the use of drugs which can selectively affect the peptidergic system (e.g. by destruction of nerve cells, blockade of receptors, inhibition of enzymatic destruction, interference with biosynthesis) and also the correlation of carotid body peptide release with chemoreceptor activity under various physiological conditions. It will also be necessary to establish whether the polypeptides which appear to be present in the carotid body are the endogenous physiologically active entities, or whether they are converted to or derived from more active forms. Most of these ideals seem unattainable at present because of lack of suitable drugs or techniques, but this situation may change rapidly.

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Excitatory action of adenosine on cat carotid chemoreceptors

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There do not appear to be any reports concerning the action of adenosine on cat carotid chemoreceptors. In view of the suggestions that adenosine may play a role in neurotransmission (e.g. Ribeiro, 1979), it seemed of interest to study the effect of adenosine on cat carotid chemosensory activity.

Experiments were performed on pentobarbitone-anaesthetized cats (42 mg kg^{-1} I.P. supplemented every 1–2 h). The animals were artificially ventilated with air and paralysed with gallamine (3 mg kg^{-1} I.V.). Chemoreceptor activity was recorded from the peripheral end of a sectioned sinus nerve (McQueen, 1977); the ganglio-glomerular (sympathetic) nerves were usually cut. Drug solutions were injected into the ipsilateral common carotid artery (I.C.) over a 2 s period.

Adenosine (0·01–100 μg I.C.) caused a rapid and marked increase of spontaneous chemoreceptor discharge (e.g. Fig. 1), the intensity, duration and onset of which was dose-dependent. Theophylline (0·1–1 mg I.C.), an adenosine antagonist (see Fredholm, 1980), decreased spontaneous chemoreceptor discharge but did not significantly alter the excitatory action of adenosine.

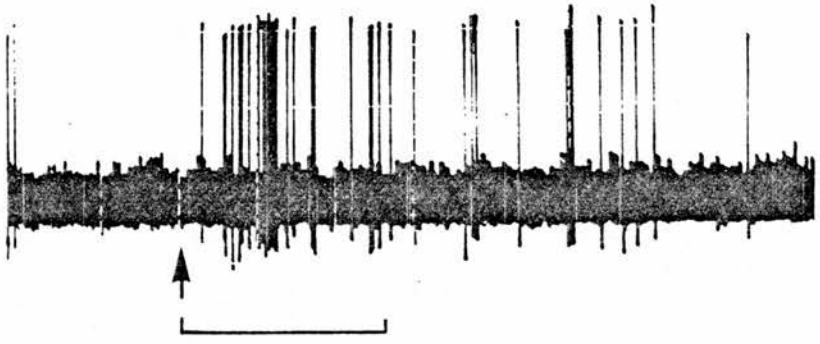

Fig. 1. Recording of a single chemoreceptor unit illustrating the response to adenosine (1 μg I.C.). Panel shows from above downwards: action potentials, injection marker and 10 s calibration.

Responses to the stimulants NaCN (2·5 or 5 μg I.C.), CO_2 (0·3 ml I.C. CO_2-saturated Locke), acetylcholine (ACh, 50 μg I.C.) and to the inhibitor, dopamine (DA, 5 μg I.C.), were determined before and after adenosine injections, and expressed as $\Delta\Sigma x$ (McQueen, 1977). Responses to NaCN and CO_2 were slightly and variably decreased after adenosine whereas both the excitatory response to ACh and the inhibitory response to DA were increased.

These results indicate that adenosine increases spontaneous chemoreceptor activity and facilitates the action of both ACh and DA on the carotid chemoreceptors.

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EFFECT OF ADENOSINE ON CAROTID CHEMORECEPTOR ACTIVITY IN THE CAT

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1 The effects of intracarotid (i.c.) injections or infusions of adenosine on chemoreceptor activity recorded from the peripheral end of a sectioned carotid sinus nerve have been studied in cats anaesthetized with pentobarbitone.

2 Adenosine injections (0.1–100 μg) caused a rapid and marked increase of spontaneous chemoreceptor discharge, the intensity, duration and onset of which was dose-dependent. Infusion of adenosine, 50 μg/min, also evoked an increase in discharge which persisted for the duration of the infusion.

3 Both theophylline (1 mg i.c.) and aminophylline (1 mg i.c.) caused short-lasting decreases in spontaneous discharge but did not prevent the excitatory effect of adenosine. Theophylline increased the excitatory action of adenosine.

4 Naloxone (400 μg i.c.) antagonized the depressant effect of morphine on chemoreceptor discharge but not the excitatory action of adenosine.

5 It is concluded that exogenous adenosine can excite the cat carotid chemoreceptors, an effect which is not prevented by theophylline in the doses studied. The physiological significance of the findings is discussed.

Introduction

There do not appear to be any reports concerning the action of adenosine on peripheral arterial chemoreceptors. In view of the suggestions that adenosine may play a role in neurotransmission (e.g. Ribeiro, 1979), it seemed of interest to study the effect of adenosine on cat carotid chemosensory activity. A preliminary account of some of this work has been presented to the Physiological Society (McQueen & Ribeiro, 1981b).

Methods

Experiments were performed on 20 cats of either sex weighing between 2.4 and 3.4 kg (median 3.1 kg). The animals were anaesthetized with pentobarbitone sodium (42 mg/kg i.p. initially, supplemented by i.v. administration of 10% of the initial dose every 1 to 2 h), artificially ventilated and paralysed by gallamine triethiodide (3 mg/kg i.v.). The ganglioglomerular (sympathetic) nerves were usually cut. Full details of the experimental techniques, including the recording of arterial blood pressure and monitoring and regulation of arterial blood gas tensions and pH, have been given previously (McQueen, 1977; Docherty & McQueen, 1978).

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Electrical activity of chemoreceptor units (1 to 6 units) was recorded from filaments of the peripheral end of a sectioned sinus nerve, passed through a pulse height (window) discriminator, and quantified with the aid of a PDP-8 computer.

A catheter was introduced via the lingual artery into the common carotid artery ipsilateral to the sinus nerve from which activity was recorded and advanced until its tip lay about 2 cm caudal to the carotid bifurcation. In some experiments a second catheter was positioned in the same common carotid artery, this time via the superior thyroid artery, and used for infusing drug solutions (0.1 ml/min).

Drugs were dissolved in modified Locke solution (McQueen, 1977) or 0.9% w/v aqueous sodium chloride (saline). Drug solutions (0.1 ml, except adenosine 100 μg which was in 0.2 ml) were injected into the common carotid artery and washed in with 0.2 ml Locke solution which had been bubbled with 5% CO_2: 95% air in a water bath at 37°C; injections were made over 2 s.

Results obtained from different experiments were pooled and expressed as the mean ± s.e. mean of the absolute values, which varied from experiment to experiment according to the number of units recorded. In order to determine whether changes observed were statistically significant, particularly when such changes were small, responses to particular drug

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doses in the different experiments were compared with the corresponding responses to the drug vehicle in the same experiments using either the Wilcoxon signed ranks test (when the number of pairs >7) or Student's paired t test (for <6 pairs, and assuming Gaussian distribution). The null hypothesis was rejected if $P<0.05$ and the difference between groups was considered statistically significant.

Drugs used were: adenosine, theophylline, aminophylline (Sigma); morphine sulphate, pentobarbitone sodium, gallamine triethiodide (May & Baker); naloxone hydrochloride (Endo).

Results

Injections of adenosine

The effects of intracarotid injection of different doses of adenosine on spontaneous chemoreceptor discharge in one experiment are illustrated in Figure 1. Discharge for each test was averaged over 10 s periods starting 10 s before and continuing for 60 s after the injection. As can be seen from either the neurograms or the averaged discharge, the main effect of adenosine was a dose-dependent increase in the frequency of discharge. No tachyphylaxis to the action of adenosine was observed. Thus, when injected at intervals of 5 min over a period of 1 h, there was no diminution of the effect on spontaneous chemoreceptor discharge.

Low doses of adenosine (0.001 to 0.01 μg) caused a decrease in chemoreceptor discharge during the first 10 s following the injection (see Figure 1), an effect which appeared to be most pronounced for the lowest dose. However, an inhibition of spontaneous discharge was also usually associated with injection of the same volume of drug vehicle, Locke solution

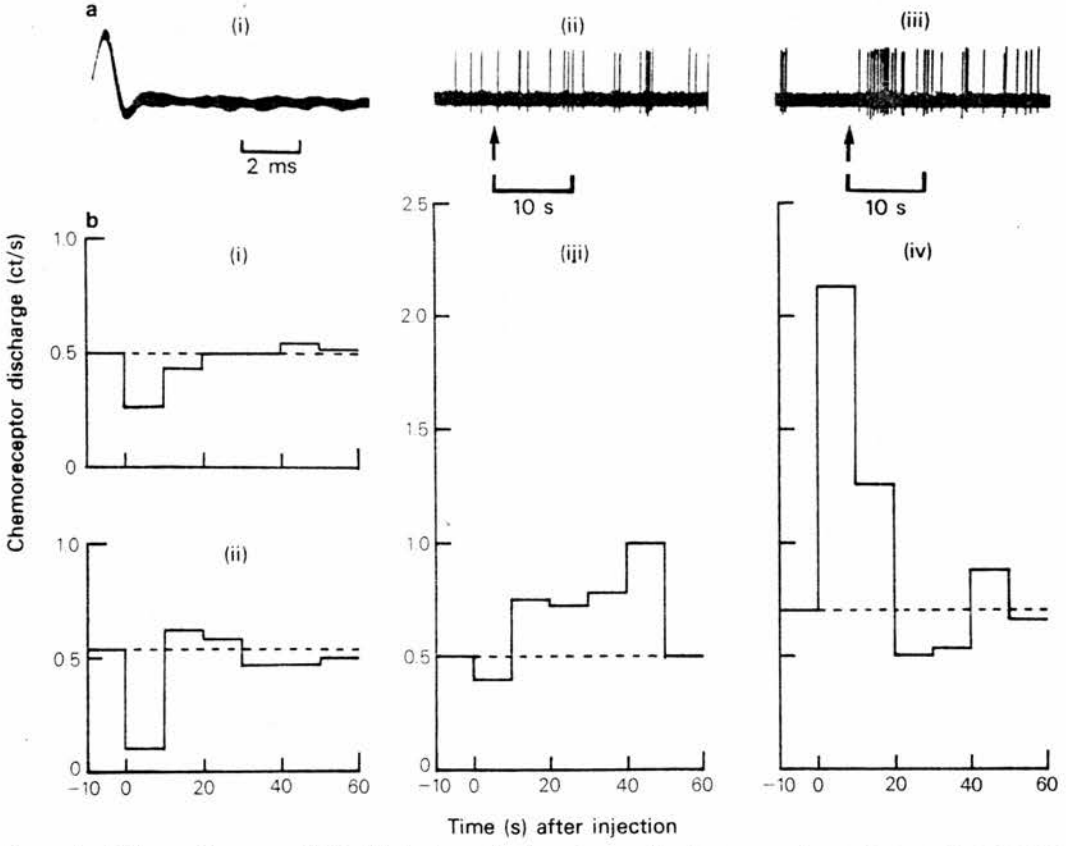

Figure 1 Effects of intracarotid (i.c.) injections of adenosine on the frequency of spontaneous chemoreceptor discharge. (a) Neurograms taken from one experiment show the increase in the discharge of a single unit (insert, i) caused by injecting (arrow) adenosine 0.1 (ii) and 1 μg (iii); in (i) the duration of the action potential is shown, the trace being of 10 consecutive superimposed spikes. (b) Responses of the chemoreceptor unit to i.c. injection of (i) 0.3 ml of the Locke solution and to adenosine: 0.001 μg (ii); 0.1 μg (iii) and 1 μg (iv). The graphs show the amplitude and duration of the responses averaged over 10 s periods following the injections.

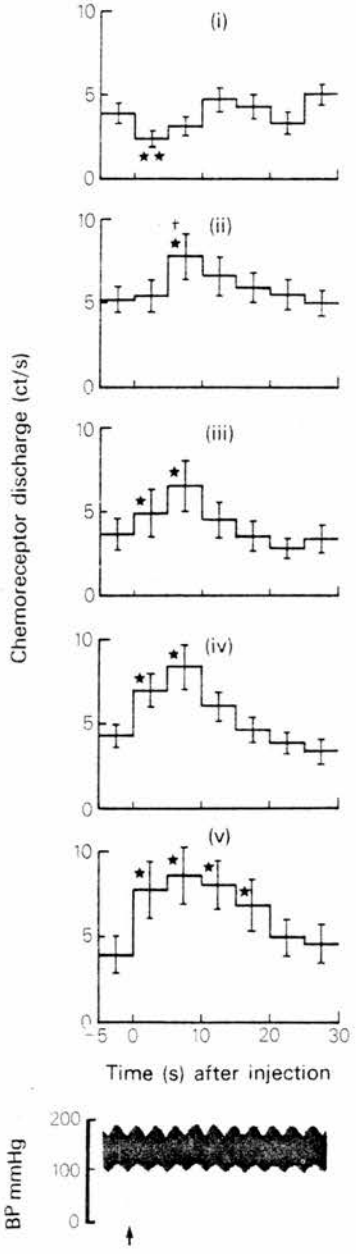

Figure 2 Effects on spontaneous chemoreceptor discharge of injecting Locke solution 0.3 ml (i) and adenosine 0.1 μg (ii); 1 μg (iii); 10 μg (iv) and 100 μg (v). Discharge was averaged over 5 s periods following the injection. Data obtained from tests in 8–10 cats were pooled and presented as the mean with vertical bars indicating s.e.mean. The lower panel shows the effect on arterial blood pressure (BP) of injecting (arrow) 100 μg of adenosine in one experiment. $^{*}P<0.05$ compared with Locke solution injections; $^{**}P<0.05$ compared with pre-injection control discharge; $^{\dagger}P>0.05$ compared with adenosine 100 μg.

(see Figures 1 and 2), so responses to the low doses of adenosine were studied in five cats (results not shown) and compared with responses to injections of Locke solution (0.3 ml). There was no significant difference ($P>0.05$) between the responses to adenosine and Locke solution in these experiments.

Data obtained from ten experiments were averaged over 5 s periods for 30 s after the injection, pooled and plotted against time (Figure 2). This quantitative evidence confirmed that the increase in both amplitude and duration of the discharge were dose-related. The peak of the discharge, averaged over 5 s periods, for all doses (0.1–100 μg) occurred between 5 and 10 s after the injections, and that caused by 0.1 μg was not significantly different from that caused by the highest dose studied, 100 μg ($P>0.05$, $n=8$ pairs). Injections of Locke solution were also made in these experiments and the results obtained are summarized in Figure 2. They confirm the transient inhibitory effect of Locke solution illustrated in Figure 1. A significant decrease in discharge occurred during the first 5 s following the injection ($P<0.05$, $n=10$, compared with the pre-injection averaged discharge). This was less marked and not statistically significant in the next 5 s ($P>0.05$), and thereafter discharge returned to the pre-injection control levels.

Results from four experiments in which both average discharge and maximum discharge, expressed as ct/s, were obtained for different doses of adenosine are compared and summarized in Table 1. The average spontaneous chemoreceptor discharge was determined in the 15 s pre-injection control period immediately preceding each injection and in the 15 s post-control period commencing 45 s after the injections. Responses to adenosine were evaluated by determining the average discharge in the period during which discharge was increased above control frequency. Apart from increasing the average discharge, adenosine also increased the maximum discharge frequency. This latter increase, however, was not so marked as that averaged over the whole period, showing that the overall increase in discharge caused by adenosine resulted from a sustained increase throughout the response rather than from a sudden transient increase, such as occurs with acetylcholine (ACh) (cf. McQueen, 1977). The duration of the adenosine response was dose-dependent, and the delay to onset of the response was inversely related to dose.

The effects obtained were not related to changes in systemic blood pressure since adenosine in the doses studied (0.001 to 100 μg) did not cause any consistent or substantial changes in arterial blood pressure, as can be seen from Figure 2. There were no changes in arterial blood gas tensions or pH following administration of adenosine.

Table 1 Effect of adenosine on chemoreceptor discharge in the cat

	Chemoreceptor discharge				Maximum discharge		Response	
	Average discharge							
Adenosine (μg) (No. of expts)	*Pre-control** (= 100%) (ct/s) Mean, range	*Effect of adenosine* (% of control) Mean, range	*Post-control*** (= 100%) (ct/s) Mean, range	*Pre-control*† (= 100% (ct/s) Mean, range	*Effect of adenosine* (% of control) Mean, range	*Post-control*‡ (= 100%) (ct/s) Mean, range	*Duration* (s) Mean, range	*Delay To onset* (s) Mean, range
0.1 (4)	6.1 5.2–6.9	156 108–184	5.1 3.9–5.8	12 11–14	135 73–181	11 8–13	8.0 6.0–11.7	7.1 3.2–10.1
1 (3)	4.9 3.6–6.2	181 172–191	5.0 3.7–7.1	10 8–12	142 70–206	11 8–12	12.7 9.0–15.4	5.4 2.1–11.3
10 (4)	4.0 1.9–5.1	209 195–218	3.6 1.3–4.7	9 5–12	175 143–196	10 6–13	13.8 12.1–14.6	4.7 2.1– 8.2
50 (3)	3.6 1.9–5.1	248 247–249	3.8 2.1–4.7	7 5–10	209 183–223	9 6–11	15.1 13.7–16.5	3.6 0.7– 8.4
100 (3)	5.5 1.8–7.6	229 221–243	5.5 2.8–6.9	13 5–17	209 180–240	12 7–15	19.8 14.3–25.4	1.7 0.5– 3.0

*Discharge averaged during the 15 s immediately before injecting adenosine.
**Discharge averaged during the 15 s period commencing 45 s after the injection, by which time the discharge had usually returned to pre-injection levels. There was no significant difference between pre- and post-control values ($P > 0.05$).
† Maximum discharge in 1 s observed during the pre-control period.
‡ Maximum discharge in 1 s observed during the post-control period.

Theophylline and aminophylline

Theophylline (1 mg) was given as an intracarotid (i.c.) injection in five cats and caused a decrease in spontaneous chemoreceptor discharge, an effect which lasted for about 30 s. The integrated response ($\Delta\Sigma x$) showed that the depression was significantly greater ($P < 0.05$) than that caused by injecting Locke solution. Although theophylline transiently decreased spontaneous chemoreceptor discharge (Figure 3), it did not prevent the excitatory action of adenosine, and as can be seen in Figure 4, the log dose-response curve to adenosine was shifted upwards and to the left which is indicative of an increase in the responses after theophylline; the response to adenosine 100 μg was statistically significantly greater after theophylline than it was before ($P < 0.05$, $n = 4$).

Injections of lower doses of theophylline (100–200 μg) did not cause any significant effect on the spontaneous discharge ($P > 0.05$, $n = 3$ pairs), and the excitatory effect of adenosine (1–100 μg) was unaltered by these doses ($P > 0.05$, $n = 3$ pairs).

Aminophylline (1 mg i.c.) was injected in one cat

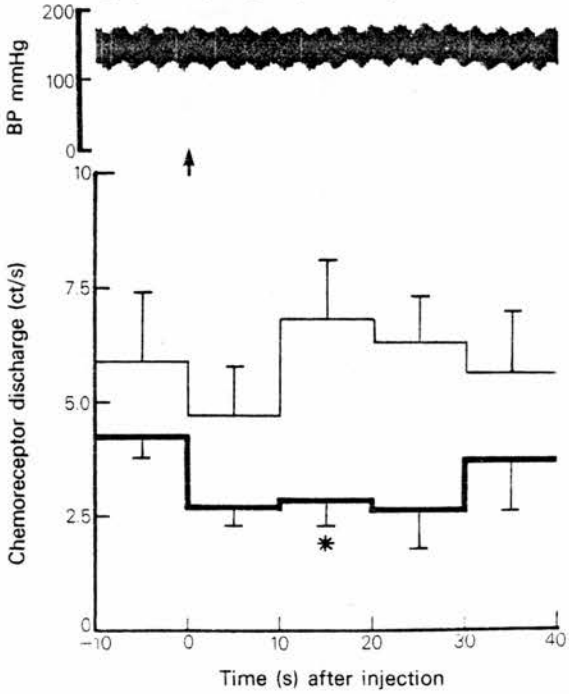

Figure 3 Effect on spontaneous chemoreceptor discharge of injecting theophylline 1 mg i.c. (heavy line, ——) compared with that of injecting Locke solution (0.3 ml) (thin line, ——). Discharge was averaged over 10 s periods following the injection. *$P < 0.05$, $n = 5$ pairs. The upper panel shows the effect on arterial blood pressure of injecting (arrow) 1 mg of theophylline in one experiment.

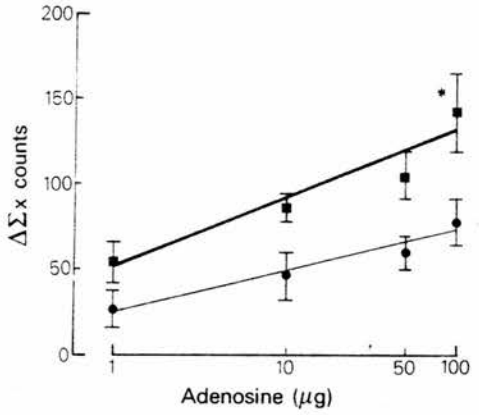

Figure 4 Dose-response data for adenosine obtained before (●) and after (■) theophylline (1 mg i.c.) in four experiments. Doses (μg i.c.) are plotted on a $\log_{10}$ scale and chemoreceptor responses expressed as the mean $\Delta\Sigma x$ for the four experiments; vertical lines show s.e.mean. $\Delta\Sigma x$ was calculated as the response during the 30 s following the injections. ($\Delta\Sigma x = \Sigma x - \bar{x}.t$, where Σx is the number of action potentials counted during the response of duration t s, a 'response' being defined as lasting from the first substantial change from background discharge frequency ($\bar{x}$ ct/s) until return to background level.) Lines were fitted to the data by the method of least squares. Averaged values ± s.e.mean (ct/s) for the pre-injection (15 s) control periods are as follows: adenosine 1 μg 4.5 ± 1.0 before theophylline, 6.5 ± 1.4 after theophylline; adenosine 10 μg 5.5 ± 1.4 before, 7.8 ± 1.7 after, adenosine 50 μg 3.5 ± 1.1 before, 5.2 ± 1.4 after; adenosine 100 μg 3.9 ± 1.7 before, 5.1 ± 2.0 after. * $P < 0.05$.

and the effect of adenosine (1 – 100 μg) tested before and after the injection. A transient depressant effect similar to that seen with theophylline was observed, and the excitatory action of adenosine was also undiminished after aminophylline.

Neither theophylline (Figure 3) nor aminophylline caused any marked changes in blood pressure and the highest dose of adenosine did not alter arterial blood pressure when injected after theophylline.

Naloxone

Four experiments were performed to investigate the influence of naloxone (0.4 mg i.c.) on chemoreceptor responses to adenosine and morphine. The adenosine results were pooled and are shown in Figure 5. No statistically significant difference was detected between the pre- and post-naloxone response ($P > 0.05$). In two of these experiments morphine (10 – 100 μg i.c.) was injected before and after naloxone (Figure 5). A chemodepressant effect of morphine was obtained in accordance with previous observations (McQueen & Ribeiro, 1980). This depressant effect of morphine was converted to an excitatory one by naloxone.

Infusions of adenosine

Adenosine, infused at a rate of 50 μg/min for 2 min in two cats, caused a sustained increase in chemoreceptor discharge, which returned to pre-infusion control levels within 30 s of stopping the infusion (Figure 6). The peak response was significantly different from the control, whether this was taken as the pre-infusion discharge or the discharge averaged during an infusion of a Locke solution (0.1 ml/min). The infusion of Locke solution did not affect spontaneous discharge (Figure 6). Adenosine infusions were not

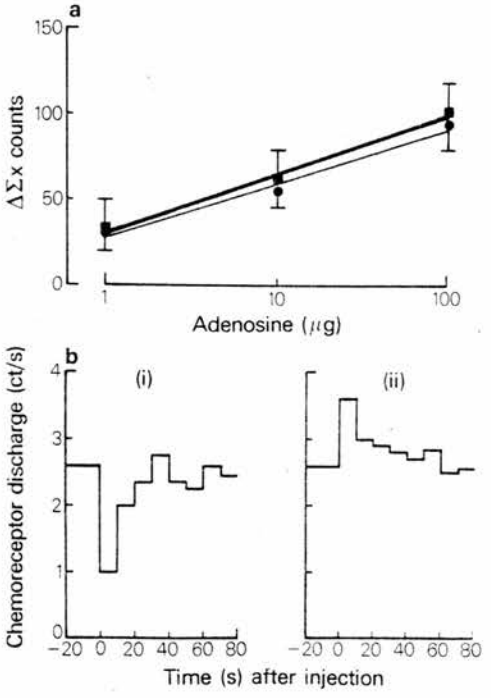

Figure 5 (a) Dose-response data for adenosine obtained before (●) and after (■) administration of naloxone (400 μg i.c.) in four experiments. Doses (μg i.c.) are plotted on a $\log_{10}$ scale and chemoreceptor responses expressed as the mean $\Delta\Sigma x$ for the four experiments; vertical lines show s.e.mean. $\Delta\Sigma x$ was calculated as the response during the 30 s following the injections. For details see Figure 4. Lines were fitted to the data by the method of least squares. Averaged values (ct/s) for the pre-injection (15 s) periods ± s.e.mean were as follows: adenosine 1 μg 4.5 ± 2.1 before naloxone, 5.7 ± 2.6 after naloxone; adenosine 10 μg 4.2 ± 1.4 before, 6.7 ± 1.5 after; adenosine 100 μg 6.5 ± 1.3 before, 5.0 ± 1.9 after. (b) Effects of morphine (100 μg i.c.) on chemoreceptor discharge before (i) and after (ii) naloxone (400 μg i.c.) in one experiment. Discharge was averaged over 10 s periods following the injection.

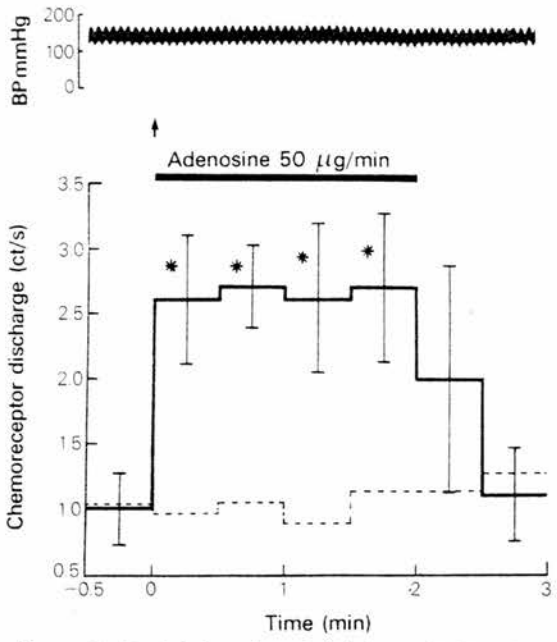

Figure 6 Pooled data from 6 infusions of adenosine (50 μg/min) in 2 cats showing the effect on spontaneous chemoreceptor discharge. The broken line represents the typical discharge during an infusion of Locke solution (0.1 ml/min). The upper panel shows a blood pressure trace recorded during one of the adenosine infusions. * $P < 0.05$.

associated with changes in arterial blood pressure, as can be seen from Figure 6.

Discussion

The results show that the predominant effect of injecting or infusing adenosine close-arterial to the cat carotid chemoreceptors is an increase in spontaneous discharge frequency.

In many preparations exogenous adenosine decreases the release of neurotransmitters (e.g. ACh (Ginsborg & Hirst, 1972; Ribeiro & Walker, 1975; Vizi & Knoll, 1976; Gustafsson, Hedqvist, Fredholm & Lundgren, 1978); noradrenaline (Hedqvist & Fredholm, 1976; Verhaeghe, Vanhoutte & Shepherd, 1977; Wakade & Wakade, 1978), dopamine (Michaelis, Michaelis & Myers, 1979) γ-aminobutyric acid (Hollins & Stone, 1978)). If adenosine exerts a similar action in the carotid body, its excitatory effect could be interpreted as being the consequence of decreased release of an inhibitory transmitter and/or modulator. Dopamine (Chiocchio, Biscardi & Tramezzani, 1966; Zapata, Hess, Bliss & Eyzaguirre, 1969) and enkephalins (Lundberg, Hökfelt, Fahrenkrug, Nilsson & Terenius, 1979; Wharton, Polak, Pearse, McGregor, Bryant, Bloom, Emson, Bisgard & Will, 1980) appear to be present in the cat carotid body and both exogenous dopamine (Zapata, 1975; Docherty & McQueen, 1978) and the enkephalins (McQueen & Ribeiro, 1980; 1981a; McQueen, 1981) depress spontaneous chemoreceptor activity. Osborne & Butler (1975) suggested that tonically released dopamine may suppress chemoreceptor discharge and although evidence which is not in agreement with their hypothesis has been obtained (e.g. Docherty & McQueen, 1978), the present results could be explained in terms of adenosine inhibiting the release of dopamine, or some other substance which is tonically active in inhibiting discharge (? enkephalins).

However, it has also been found that adenosine can excite nerve cells. For example, Siggins, Gruol, Padjen & Forman (1977) showed that adenosine depolarizes neurones of explanted amphibian sympathetic ganglia, and Bleehen & Keele (1977) described algogenic actions of adenosine on the human blister base. It appears that adenosine sensitizes the carotid chemoreceptors to ACh and dopamine (McQueen & Ribeiro, 1981b) which might be construed as evidence that adenosine acts directly on the postsynaptic component of the carotid body chemosensory synapse, i.e. on the sensory nerve ending. An adenylate cyclase-cyclic AMP system is apparently located in the sinus nerve endings (Fitzgerald, Rogus & Dehghani, 1977) and it may be that adenosine interacts with this in a manner similar to that described for the brain (e.g. Davies, Taylor, Gregson & Quinn, 1980). Further investigation is needed to explore this possibility.

Theophylline appears unable to prevent activation of P_2-purinoceptors. These receptors, as so far described, seem to be localized post-junctionally (see Burnstock, 1978). De Mey, Burnstock & Vanhoutte (1979) found that in the canine saphenous vein, theophylline antagonizes the inhibitory effect of ATP on neurogenic responses but not its direct contractile effect. According to these authors this suggests the presence of both inhibitory presynaptic (P_1) and excitatory postsynaptic (P_2) receptors. In the present study the excitatory effect of adenosine was unaffected by low doses of theophylline (0.1–0.2 mg) but potentiated by a slightly higher dose (1 mg), a finding that is compatible with a P_2-excitatory postjunctional effect. However, the potentiation might also have resulted from theophylline antagonizing a chemodepressant component of the adenosine response, which might normally be masked by the excitatory component, or from mechanisms having in common the ability to increase the cyclic AMP concentration (e.g. theophylline by inhibiting phosphodiesterases, and adenosine by activating the adenylate cyclase).

It has also been proposed that the inhibitory action of morphine may involve the release of adenosine as an intermediary (Sawynok & Jhamandas, 1976; Stone & Perkins, 1979), and it has been shown that morphine and enkephalins can inhibit spontaneous carotid chemosensory discharge in the cat (McQueen & Ribeiro, 1980; 1981a; McQueen, 1981). However, naloxone reduces the inhibitory effect of morphine without reducing the excitatory response to adenosine, so the effects of adenosine do not appear to result from actions on naloxone-sensitive opiate receptors in the carotid body.

Adenosine can cause vasodilatation (see Berne, Foley, Watkinson, Miller, Winn & Rubio, 1979) and we cannot, therefore, preclude the possibility that adenosine was changing blood flow in the carotid body and thereby, perhaps, altering chemoreceptor discharge, even though it had little overall effect on the systemic blood pressure. However, other vasodilators (e.g. sodium nitrite, sodium nitroprusside: Docherty, 1980) tend to cause a delayed decrease in discharge, which is the opposite of the main effect obtained following adenosine injections in the present experiments. We consider it unlikely that vascular effects are responsible for the greater part of the response to adenosine, but would wish to investigate this with *in vitro* carotid body studies.

Adenosine is released into the circulation by a number of physiological and pathophysiological processes (e.g. ischaemia; see Winn, Rubio & Berne, 1979), and a correlation between adenosine concentration and oxygen supply in rat brain has been suggested (Rubio, Berne, Bockman & Curnish, 1975). In the present study, low doses of adenosine modified chemoreceptor discharge and it seems reasonable to speculate that adenosine might influence events taking place in the carotid body, perhaps by modulating any putative transmitter(s) which may be released within the carotid body complex, and/or by directly activating sensory nerve endings.

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